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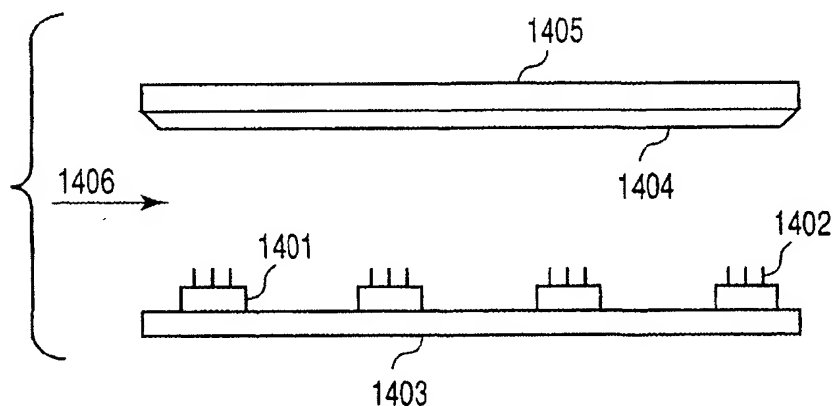
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(54) **Nucleic acid detection sensor**

(57) A nucleic acid detection sensor comprises a plurality of nucleic acid chain fixed electrodes (1401) to which a probe nucleic acid chain is fixed, and a counter

electrode (1404) which is arranged opposite to the nucleic acid chain fixed electrode, and a current flowing between the counter electrode and the nucleic acid chain fixed electrode.

FIG. 14A



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Description

[0001] The present invention relates to a nucleic acid detection sensor which electrochemically detects whether a target nucleic acid chain in a test liquid has a specific base sequence.

[0002] Recently, a genetic test technique with a nucleic acid chain fixed array (DNA array) is made attention as a nucleic acid detection sensor (see "Beattie et al. 1993, Fodor et al. 1991, Khrapko et al. 1989, Southern et al. 1994").

[0003] DNA array indicates the array of a glass and a silicon with several cm squares where DNAs with different 10^1 to 10^5 kinds of arrays is fixed. The signed test liquid gene is reacted with a fluorescent dye and a radioisotope (RI), etc. on the array, or the unsigned test liquid gene and the compound of the sign oligonucleotide are reacted by the sandwich hybridization. When a complementary array to the DNA on the array exists in the test liquid, the signal (fluorescent intensity and RI intensity) which is derived from the sign by a specific part on the array is obtained. If the arrangement and the position of the fixed DNA are known beforehand, the base sequence which exists in the test liquid gene can be easily checked. Since a lot of information on the base sequence with the small amount sample can be obtained, DNA array is expected very much not only in the gene detection technique and also in the sequence technical (see "Pease et al. 1994, Parinov et al. 1996").

[0004] There are a fluorescent detection method, RI intensity detection method, and an electrochemical detection method, etc. as a technique which detects the nucleic acid which is combined with the nucleic acid detection sensor. A sign of the sample gene and a complex system are not required in an electrochemical technique. Therefore, the miniaturization of the system can be expected. In addition, since the electrode used, the electrochemical technique has an advantage that an electric reactive control can be easily performed.

[0005] Especially, among the nucleic acid detection sensors which use an electrochemical technique, the sensor having a DNA array configuration in which a plurality of electrodes where a different probe nucleic acid chain is fixed are arranged in a X-Y matrix is expected as an extremely useful technical which can detect many kinds of nucleic acids with a short time. However, the equal voltage should be applied to a lot of nucleic acid chain fixed electrodes in this sensor. Therefore, this sensor has the problems such as the circuit configuration is complex, and the response speed and accuracy are not sufficient.

[0006] An object of the present invention is to provide a nucleic acid detection sensor which can detect many kinds of nucleic acids with high-speed and high-accuracy.

[0007] A first nucleic acid detection sensor according to the present invention is characterized by comprising: a plurality of nucleic acid chain fixed electrodes to which

a probe nucleic acid chain is fixed; and a counter electrode which is arranged opposite to the nucleic acid chain fixed electrode, wherein a current flowing between the counter electrode and the nucleic acid chain fixed electrode. Since the nucleic acid chain fixed electrode and the counter electrode are opposing arranged, the measurement of high accuracy can be promptly performed by the decreased amount of the test liquid.

[0008] A second nucleic acid detection sensor according to the present invention by comprising: a plurality of nucleic acid chain fixed electrodes to which the probe nucleic acid chain is fixed; a counter electrode, a current flowing between each of the nucleic acid chain fixed electrodes and the counter electrode; and a reference electrode provided for each of the nucleic acid chain fixed electrodes, configured to make a voltage between the nucleic acid chain fixed electrode and the counter electrode constant. The measurement sensitivity is improved since the reference electrode is arranged for each nucleic acid chain fixed electrode.

[0009] A the third nucleic acid detection sensor according to the present invention by comprising: a plurality of nucleic acid chain fixed electrode, to which a probe nucleic acid chain is fixed, arranged in a matrix; a plurality of scanning lines configured to select the plurality of nucleic acid chain fixed electrodes one by one; a plurality of signal lines configured to transmit a measurement signal from the plurality of nucleic acid chain fixed electrodes; a plurality of switching elements connected with the plurality of signal lines; and an A/D converter connected with the plurality of switching elements. The configuration becomes simple since only one A/D converter is prepared because the output line of the signal is shared with the switching element.

[0010] This summary of the invention does not necessarily describe all necessary features so that the invention may also be a sub-combination of these described features.

[0011] The invention can be more fully understood from the following detailed description when taken in conjunction with the accompanying drawings, in which:

FIG. 1 is a schematic diagram which shows a nucleic acid detection chip according to the embodiment of the present invention in which a plurality of electrodes are arranged;

FIG. 2 is a figure, which shows an arrangement of an electrode in a nucleic acid detection chip according to the embodiment of the present invention and the signal line;

FIG. 3 is a figure, which shows an another arrangement of an electrode in a nucleic acid detection chip according to the embodiment of the present invention and the signal line;

FIG. 4 is a figure, which shows an another arrangement of an electrode in a nucleic acid detection chip according to the embodiment of the present invention and the signal line;

FIG. 5 is an expansion figure of a unit division of a nucleic acid detection chip according to the embodiment of the present invention where the plurality of electrode is arranged;

FIG. 6 is an expansion figure of a unit division of a nucleic acid detection chip according to the embodiment of the present invention where the plurality of electrode is arranged;

FIG. 7 is an expansion figure of a unit division of a nucleic acid detection chip according to the embodiment of the present invention where the plurality of electrode is arranged;

FIG. 8A to FIG. 8C are figures which show the nucleic acid detection chip which can be installed in the nucleic acid detection system;

FIG. 9A and FIG. 9B are figures which show a nucleic acid detection chip which can be attached to the nucleic acid detection system;

FIG. 10 is a figure, which shows a nucleic acid detection system to which the nucleic acid detection chips are arranged;

FIG. 11A and FIG. 11B are figures which show the nucleic acid detection chip according to the embodiment of the present invention stored in the container;

FIG. 12 is a figure, which shows the substrate to which the nucleic acid detection chip according to the embodiment of the present invention should be installed;

FIG. 13A and FIG. 13B are figures which show a nucleic acid detection chip according to the embodiment of the present invention stored in the container;

FIG. 14A to FIG. 14D are figures which compares a nucleic acid detection chip according to the embodiment of the present invention in which electrodes are arranged at opposed positions and a conventional nucleic acid detection chip in which electrodes are not arranged at opposed positions; FIG. 15 is a figure, which shows an example of the circuit applied to the second nucleic acid detection chip according to the embodiment of the present invention;

FIG. 16 is a figure, which shows another example of the circuit applied to the second nucleic acid detection chip according to the embodiment of the present invention;

FIG. 17 is a figure, which shows another example of the circuit applied to the second nucleic acid detection chip according to the embodiment of the present invention;

FIG. 18 is a figure, which shows the configuration of the nucleic acid detection chip according to the embodiment of the present invention in which electrodes are arranged at the opposite positions;

FIG. 19 is a figure, which shows a wiring in the nucleic acid detection chip according to the embodiment of the present invention in which electrodes

are arranged at the opposite positions;

FIG. 20 is a figure, which shows the signal and the output signal to apply the voltage to each unit division; and

FIG. 21 is a figure, which shows a wiring in a nucleic acid detection chip according to the embodiment of the present invention in which electrodes are arranged at the opposite positions.

[0012] The nucleic acid detection sensor according to embodiment of present invention is characterized by comprising each of the following configurations.

(1) The nucleic acid chain fixed electrode and the counter electrode are oppositely arranged.

(2) The reference electrodes are arranged for each cell.

(3) The output line of the signal is shared with the switching element.

[0013] In the specification, in the nucleic acid detection sensor according to the present invention to which a plurality of nucleic acid chain fixed electrodes is arranged, a "nucleic acid detection cell" means the unit division (unit cell) which comprises a couple of a nucleic acid chain fixed electrode and a counter electrode.

[0014] The probe nucleic acid chain is fixed to hybridize the target nucleic acid chain in the test liquid to the nucleic acid chain fixed electrode. The nucleic acid chain fixed electrode functions as an action electrode in the nucleic acid detection sensor according to the present invention. A "probe nucleic acid chain" indicates the nucleic acid chain fixed (combined) to the nucleic acid chain fixed electrode. A "target nucleic acid chain" is a nucleic acid chain which has a complementary base sequence for said probe nucleic acid chain and reacts the hybridization with said probe nucleic acid chain, and means the nucleic acid chain included in the test liquid.

[0015] The counter electrode functions as an auxiliary electrode to flow the current which flows between nucleic acid chain fixed electrodes. In addition, the measurement method of the nucleic acid detection cell of the nucleic acid detection sensor according to the present invention may be duplex system which uses the nucleic acid chain fixed electrode and the counter electrode, applies an arbitrary voltage between the nucleic acid chain fixed electrode and the counter electrode, and detects an electrochemical change generated between both electrodes. The measurement thereof may be an electrochemical measurement using triode system to connect a reference electrode with the nucleic acid chain fixed electrode and the counter electrode of the duplex system. Since the current flows to the counter electrode in the duplex system, there is a disadvantage that the concentration of the carrier in the electrode/interface, which determines the potential of the counter electrode, changes and the potential, which becomes a reference, changes. On the other hand, in triode system, the po-

tential (potential of the reference electrode) which becomes a reference does not change, since the current flows between the nucleic acid chain fixed electrode and the counter electrode, the current hardly flows between the nucleic acid chain fixed electrode and the reference electrode and between the counter electrode and the reference electrode, and the voltage is applied between the nucleic acid chain fixed electrode and the counter electrode so that the desired potential may be applied to the reference electrode.

[0016] The finding of the target nucleic acid chain or the probe nucleic acid chain is obtained with the nucleic acid detection sensor according to the present invention as follows. The voltage is applied between the nucleic acid chain fixed electrode and the counter electrode in the nucleic acid detection cell in the existence of the test liquid including the nucleic acid chain. After the hybridization is occurred between the target nucleic acid chain and the probe nucleic acid chain, an electrochemical change generated between electrodes is detected. An electrochemical change is generated between electrodes if the target nucleic acid chain is hybridized with the probe nucleic acid. Therefore, if the corresponding change is detected whether the probe nucleic acid chain or the target nucleic acid chain has a specific base sequence can be detected. It is preferable that an electrochemical change generated between electrodes because of the above-mentioned hybridization is a current change which adds two chain recognition bodies in the test liquid, and derives from the two chemical change of the chain recognition body. As a result, the measurement can be performed with ease and high accuracy.

[0017] The nucleic acid chain having a known base sequence is used as a probe nucleic acid chain to be fixed to the nucleic acid chain fixed electrode. Whether the target nucleic acid chain, which reacts the hybridization in the test liquid with said probe nucleic acid chain exists may be detected. The nucleic acid chain with an unknown base sequence as a probe nucleic acid chain to be fixed to the nucleic acid chain fixed electrode is used. Whether or not the target nucleic acid chain which reacts the hybridization in test liquid with said probe nucleic acid chain exists is detected by containing the nucleic acid chain having a known base sequence in test liquid. Thus, the finding of the array of the probe nucleic acid chain with the unknown base sequence can be obtained.

[0018] Typically, a different kind of the probe nucleic acid chain is fixed to each of the plurality of nucleic acid chain fixed electrodes. To supply different samples in each cell and perform the check of several samples at a time, the same kind of probe nucleic acid chain may be fixed.

[0019] Since one nucleic acid chain fixed electrode is arranged to the each nucleic acid detection cell, the finding of the array of the target nucleic acid chain or the probe nucleic acid chain is obtained. By checking the target nucleic acid chain hybridize to the nucleic acid

detection cell. Therefore, it is preferable to arrange a switching circuit, a decoder circuit, or, a timing circuit to apply the electric signal to each nucleic acid chain fixed electrode of each cell, a circuit which outputs an electric signal from each nucleic acid chain fixed electrode, and a switching circuit to output an electric signal from each nucleic acid chain fixed electrode to an external device to operate the each nucleic acid detection cell independently.

[0020] The plurality of scanning lines are connected with the circuit such as the switching circuits to apply an electric signal to each nucleic acid chain fixed electrode of each cell. The signal to turn on the transistor, desirably, a switching element such as a thin film transistor, arranged between the nucleic acid chain fixed electrode and the signal line is applied to the scanning line. A "signal line" means a conductor line which transfers the signal specifying an electric change from the nucleic acid chain fixed electrode. The nucleic acid chain fixed electrode is an action electrode in this specification. The voltage is applied to the nucleic acid chain fixed electrode when the switching element is turned on according to the signal from the scanning line, then an electrochemical change is occurred. The change in the voltage (and/or current) by the electrochemical change is transmitted by the signal line. It is preferable to use the matrix system used to display the liquid crystal in the control of the plurality of electrodes, particular. Furthermore, it is preferable that there is an active matrix system to use the MOSFET. The scanning circuit of the MOS image sensor type can be used.

[0021] FIG. 1 shows the structure of the typical nucleic acid detection sensor which comprises the circuit to apply the voltage to each nucleic acid chain fixed electrode. FIG. 1 shows a case of measurement system of a two-electrode system. The switching element 103 connected with each nucleic acid chain fixed electrode 102 turns off and on by the signal being output to the scanning line driving circuit 107 in order to drive the scanning line 104 from the timing circuit 106 one by one in the nucleic acid detection sensor of FIG. 1. The counter electrode is connected with the power supply (not shown in the figure) through the pontiostat circuit 110. The voltage is applied to the nucleic acid chain fixed electrode 102 and counter electrode 101 when the switching element 103 opens and closes one by one. As a result, the nucleic acid (not shown in the figure) which hybridizes to the nucleic acid chain fixed electrode 102 can be detected electrochemically. An electrochemical change is transferred to the signal detection circuit 109 through the signal line 105 and is detected.

[0022] The signal line is preferably coated with the insulation material excluding the contact with the switching element as shown in FIG. 2. FIG. 2 is side figure when the cell (rectangle with dotted line) for the nucleic acid detection in the nucleic acid detection sensor of FIG. 1 is cut along parallel to the scanning line to cross the nucleic acid chain fixed electrode and the counter

electrode. In FIG. 2, the signal line 202 coated by the insulation film 203, the nucleic acid chain fixed electrode 204, and the counter electrode 205 are arranged on the insulation substrate 201. Since the signal line 202 is soaked in the test liquid, the signal line 202 is coated by the insulation film 203 excluding the contact of the signal line 202 and the switching element (not shown in the figure: contact).

[0023] The arrangement of the signal line coated with the insulation material, the switching element, and the electrode is not limited to the arrangement of FIG. 2, but may be arbitrary arrangement. FIG. 3 is an example of such an arrangement, and switching elements 304 and 305 are respectively put under the nucleic acid chain fixed electrode 302 and the counter electrode 303 (or, reference electrode) arranged on the insulation substrate 301. The switching elements 304 and 305 are coated by the insulation films 306 and 307 which exist respectively on both sides thereof. Since the contact part of the signal line (not shown in the figure) and the switching element is not contacted with the test liquid 308 even when the test liquid 308 is added on the nucleic acid chain fixed electrode and the counter electrode if the switching element is put under each electrode as shown in FIG. 3, it is excellent in insulation properties. The arrangement of FIG. 4 also has the excellent insulation properties, since the switching element is put under each electrode as well as FIG. 3. But, the point in which the switching element is exposed to the rear surface of the substrate differs from the arrangement of FIG. 3.

[0024] It is preferable that each electrode which constructs the nucleic acid detection cell is formed on the insulation substrate. As a material of the insulation substrate, for example, inorganic insulation materials such as glass, fused silica, silicon, alumina, sapphire, forsterite, and silicon carbide, silicon oxide, and silicon nitride, or, organic materials such as polyethylene, ethylene, polypropylene, polyisobutylene, polyethylene terephthalate, no saturation polyester, include fluorine resin, polyvinyl chloride, polyvinylidene chloride, polyvinyl acetate, polyvinyl alcohol, polyvinyl acetal, acrylic resin, polyacrylonitrile, polystyrene, acetal, polycarbonate, polyamide, phenol resin, urea resin, epoxy resin, and melamine resin, styrene acrylonitrile copolymer, acrylonitrile butadiene styrene copolymer, silicon resin, polyphenylene oxide, and polysulfone can be used, but the material is not limited to these materials.

[0025] It is preferable that each electrode and the circuit, etc. are separated by the insulation material. The insulation material used by the present invention is not limited, but it is preferable to use photo polymer, and a photoresist material. The photoresist for an optical exposure, the photoresist for the far ultraviolet, and the photoresist for X-ray and the photoresist for the electron beam are used as a resist material. The photoresist for an optical exposure includes cyclized rubber, the polycinnamic acid, and the novolac resin as a main raw ma-

terial. Cyclized rubber, phenol resin, polymethyl isopropenyl ketone (PMIPK), and polymethyl methacrylate (PMMA), etc. are used for the photoresist for the far ultraviolet. The material of the description in the "Thin Film Handbook" (Ohmsha, Ltd.) in addition to COP and the metal acrylate can be used for resist for X-ray. In addition, the material of the description can be used for the above-mentioned document such as PMMA in resist for the electron beam. It is preferable that the resist used here is 1 mm or less and more than 100Å. It becomes possible to make the area constant by covering the electrode with the photoresist, and performing the lithography. As a result, the fixed amount of the probe nucleic acid chain becomes uniform between each electrode, and an excellent measurement in reproducibility is enabled. Conventionally, it is general to remove the resist material finally, but the resist material may be used as a part of the electrode without removing in the nucleic acid chain fixed electrode. In this case, it is necessary to use a high water-proof material for the resist material to be used. A material excluding the photoresist material may be used as the insulation layer formed on the upper portion of the electrode. For example, oxide, nitride, carbide, and other alloy of Si, Ti, Al, Zn, Pb, Cd, W, and Mo, Cr, Ta, and Ni, etc. may be used. After forming a thin film by using a sputter, an evaporation or a CVD etc. from these materials, the electrode exposure part is patterned by the photo lithography and the area is constantly controlled.

[0026] Preferably, 10^1 to 10^5 nucleic acid chain fixed electrodes are arranged on the insulation substrate. The material of a desirable nucleic acid chain fixed electrode is gold, and other materials can be used, for example, metal single substance such as alloy of gold, silver, platinum, mercury, nickel, palladium, silicon, germanium, gallium, and tungsten and alloy thereof, or, carbon etc. such as graphite and glassycarbon, or, oxide and compound thereof may be used. These electrodes can be manufactured by a plating, a print, a sputter, and an evaporation, etc. When the evaporation is performed, the electrode film can be formed with the resistive heating method, the high frequency heating method, and the electron beam heating method. The electrode film can be formed by a DC two poles sputtering, a bias sputtering, an asymmetric AC sputtering, a gettering sputtering, and a high frequency sputtering when the sputtering is used. Here, when the gold is used for the electrode, the orientation index in a (111) plane of the crystal structure of the gold is important. The orientation index is obtained from the following equations by the method of Willson.

Orientation index (hkl)

$$= I_{F(hkl)} / I_{FR(hkl)}$$

Hkl; plane index

$IF_{(hkl)}$: Relative intensity in (hkl) plane
 $IFR_{(hkl)}$: $IF_{(hkl)}$ as standard gold described in ASTM card

[0027] Here, in case of the nucleic acid chain fixed electrode for the nucleic acid chain detection, it is required that the orientation index is one or more, in addition, it is preferable that the orientation index is two or more. It is also valid to heat the substrate to improve the orientation at the evaporation or the sputtering. It is preferable that the heating temperature is a range of 50°C to 500°C, though the heating temperature is not limited. It becomes possible to uniformly control the nucleic acid chain fixed amount by controlling the orientation. When the above-mentioned electrode material of the gold etc. are evaporated or sputtered to the substrate such as the glass, it becomes possible to form a stable electrode layer by existing titanium or chrome, copper, nickel, and alloy thereof solely or by combining between the substrate and the gold as a bonding layer.

[0028] The shape of the nucleic acid chain fixed electrode is not the limited, and the shapes shown in FIG. 5 to FIG. 7 are preferable. It is advantageous since the contact area of the nucleic acid fixed electrode and the counter electrode (or, reference electrode) is large if the shapes of FIG. 6 and FIG. 7 are used. FIG. 5 to FIG. 7 are figures which expand the nucleic acid detection cell (rectangle of dotted line in FIG. 1). As well as FIG. 1, the nucleic acid chain fixed electrodes 501, 601 and 701 are connected with the signal lines 505, 605 and 705 through the switching elements 503, 603 and 703, respectively. The counter electrodes (or, reference electrode) 502, 602 and 702 is arranged in the neighborhood of the nucleic acid chain fixed electrodes 501, 601 and 701.

[0029] It is preferable to activate the surface of the electrode to fix the probe nucleic acid chain to the nucleic acid chain fixed electrode. Activation can be performed by the potential sweep in the sulfuric acid solution. Activation can be performed even by the mixed acid and the aqua regia, etc. The material which constructs the probe nucleic acid chain is not limited, but DNA, RNA, PNA, and other nucleic acids analog can be used.

[0030] The method of fixing the probe nucleic acid chain is not especially limited. For example, it is possible to perform fixation easily by using the bond of the thiol radical and gold which are introduced into the probe nucleic acid chain. Additionally, it is possible to perform fixation by physical adsorption, chemical adsorption, the canal bond, embedding, and the covalent bond, etc. The condensation medicines such as the biotin-avidin bonds and carbodiimides can be used. Fixation can be facilitated by modifying the surface of the electrode with the molecule having the functional group beforehand in these cases. In addition, to control non-peculiar adsorption of a nucleic acid to the surface of the electrode and the intercalators etc., it is preferable to cover the surface of the electrode with the mercaptan such as mercap-

toethanols and lipids such as stearyl amides

[0031] Hereinafter, the method of fixing the probe nucleic acid chain to the nucleic acid chain fixed electrode which consists of gold will be described as an example. After washing by the demineralized water, the electrode performs the activation processing. The sulfuric acid solution of 0.1 to 10 mmol/L is used for activation. Potential is scanned in this solution within the range of 1 v/s to 100,000 v/s within the range of -0.5 to 2V (vs Ag/AgCl). As a result, the surface of the electrode is activated to the state that the probe nucleic acid chain can be fixed. The thiol radical is introduced to 5' or 3' end of the probe nucleic acid chain used for fixation. The probe nucleic acid chain made a thiol dissolves to the solution of the reducer such as DTT immediately before fixation, and removes DTT by the extraction operation with the gel filtration or the acetic acid ethyl etc. immediately before use. Fixation is very easy. The probe nucleic acid chain is dissolved in the buffer liquid within the range of pH5 to pH10 as becoming within the range of 1 ng/mL to 1 mg/mL within the range of ion intensity of 0.01 to 5. The activated electrode is soaked. The reaction of fixation is performed for about ten minutes to around one evening within the range of 4 to 100°C.

[0032] It is preferable to store the electrode after fixing the probe nucleic acid chain in the condition where the nucleic acid decomposition ferment (nuclease) does not exist, and by shading if possible. However, when it is short-term, it is possible to be stored in the wet condition. It is preferable that the composition of the medium is a composition of the liquid to which the hybridization reacts, Tris-EDTA buffer liquid, or demineralized water. It is preferable that the storage temperature is 4°C or less, preferably, - 20°C. When the nucleic acid chain fixed electrode to which the probe nucleic acid chain is fixed is stored at a long term, it is preferable to store in a dry condition. It is possible to perform dry with freeze-drying and air drying, etc., though the method of keeping to dry is not especially limited. It is preferable that it is an inert gas, nitrogen, dry air such as argon or a vacuum, though gas phase of dry is not especially limited.

[0033] The operativeness of the test can be raised by putting the sign or the bar code on each electrode.

[0034] The probe nucleic acid chain can be fixed comparatively easily with a device of fixation which is called DNA spotter and DNA arrayer at the time of fixing the probe nucleic acid chain on the electrode. In this case, it is preferable to use the spotter of an ink jet system or an electrostatic system to prevent the surface of the electrode from being damaged. It is also possible to synthesize the nucleic acid chain directly on the surface of the electrode.

[0035] One or more counter electrodes are arranged on the nucleic acid detection sensor according to the present invention. When a single counter electrode is arranged, the plurality of nucleic acid chain fixed electrodes commonly use a single counter electrode.

[0036] If the desired voltage can be applied to the nu-

cleic acid chain fixed electrode, the distance of the nucleic acid chain fixed electrode and the counter electrode is not especially limited. It is preferable to arrange the distance within 1 cm, for example, to make the response speed fast.

[0037] It is desirable to arrange the counter electrode to become an equal distance from all the nucleic acid chain fixed electrodes in order to apply an equal voltage to all the nucleic acid chain fixed electrodes.

[0038] The material is not especially limited to the material used for the counter electrode, and the material used by the nucleic acid chain fixed electrode can be used.

[0039] When the nucleic acid detection sensor according to the present invention is measured by the triode system, the reference electrode is arranged. The silver/silver chloride electrode, mercury/mercury chloride electrodes, etc. can be used as a reference electrode, but other arbitrary electrodes can be used.

[0040] It is general to arrange the counter electrode or the reference electrode in the same substrate as the nucleic acid chain fixed electrode, but the counter electrode or the reference electrode may be arranged in the part excluding this.

[0041] Since the measurement accuracy is improved, it is desirable that a shape of reference electrode is a shape of enlarging the surface area and preventing the flow of test liquid from obstructing, though the shape thereof is not especially limited. For example, electrodes suit such a condition by making the reference electrode and the nucleic acid chain fixed electrode a comb shape in which electrodes thereof are engaged mutually.

[0042] It is preferable that the nucleic acid detection sensor according to the present invention configures the nucleic acid detection system. The corresponding system has a basic configuration comprising one or a plurality of substrates on which the plurality of nucleic acid detection cells are formed, the container which is an airtight container to hold the substrate and has at least one or more openings to transfer the liquid and a space for storing the liquid, and a terminal to connect with an external equipment.

[0043] On the system, it is preferable to comprise a circuit to apply an electric signal to the counter electrode and the nucleic acid chain fixed electrode, a switching circuit to apply an electric signal to each counter electrode and each nucleic acid chain fixed electrode, a circuit which outputs an electric signal from each nucleic acid chain fixed electrode, a switching circuit to output an electric signal from each nucleic acid chain fixed electrode outside, a power supply, a potentiostat, and a the waveform generation device. To the system, it is preferable to integrate circuits such as a decoder circuit to output an electric signal to the MOSFET switching element at specific position arranged in matrix and a nucleic acid chain fixed electrode, a switching circuit, a timing circuit, memory, an A/D converter, a waveform generation device, a power supply, a potentiostat, and an

electric signal detection circuit on one sensor.

[0044] A nucleic acid extraction mechanism, a nucleic acid refinement mechanism, and a nucleic acid amplification mechanism, etc. can be integrated on the nucleic acid detection system. If the nucleic acid detection system comprising these mechanisms is used, all series of operations of the extraction, amplification, and the detection, etc. of a nucleic acid can be automatically performed.

[0045] In FIGS. 8A to 8C show an example of the nucleic acid detection sensor which can construct the nucleic acid detection system.

[0046] The nucleic acid detection sensor of FIGS. 8A to 8C comprises a plurality of scanning lines 801, signal lines 802 arranged to cross the scanning lines 801, switching elements 803 such as thin film transistors arranged to each intersection in the scanning lines 801 and the signal lines 802, nucleic acid chain fixed electrodes 804 connected with the switching elements 803, a scanning line driving circuit 805 to drive each scanning lines 801, a first substrate 807 (FIG. 8A) on which a signal line driving circuit 806 to drive each signal line 802 is arranged, and a second substrate 809 (FIG. 8C) on which each counter electrode 808 is arranged. Each counter electrode 808 is connected with the potentiostat (not shown in the figure). Though only one nucleic acid chain fixed electrode is written in FIG. 8, actually, one nucleic acid chain fixed electrode is arranged respectively on the rectangular of each inside of the surround to two adjacent scanning line and two adjacent signal lines.

[0047] In order to detect the target nucleic acid in the test liquid, the driving signal is transmitted from the scanning line driving circuit 805 to the switching element 803, after the test liquid is injected into the space which lies between the first substrate 807 and the second substrates 809. The switching element 803 is turned on by the driving signal output from the scanning line driving circuit 805, and the nucleic acid chain fixed electrode 804 and signal line 802 are electrically connected. The voltage is applied between the nucleic acid chain fixed electrode 804 and the counter electrode 808 when the nucleic acid chain fixed electrode 804 and signal line 802 are electrically connected. As a result, for example, the stuff such as the intercalators inserted in the target nucleic acid hybridized to the nucleic acid chain fixed electrode 804 is oxidized. The current generated by the oxidation flows to the pad 810 provided to one end of the signal line 802 through the signal line 802, and is detected and measured with an external equipment for the current detection connected with the pad 810.

[0048] In the nucleic acid detection sensor of FIG. 8, a nucleic acid detection part 811, a scanning line driving circuit 805, and a signal line driving circuit 806 are formed on the first substrate 807 integrally, and the nucleic acid detection sensor is installed to the nucleic acid detection system comprising the signal detection part and is used.

[0049] In the shape shown in FIG. 9A and FIG. 9B, the counter electrode 905 may be connected with scanning lines 901 electrically and may be arranged in the vicinity of the nucleic acid chain fixed electrode. In FIG. 9A and FIG. 9B, the counter electrode 905 has a comb shape each of whose branches are branched to three, and is arranged so as to mutually engage with the nucleic acid chain fixed electrode 905 having the same shape thereof.

[0050] Though the nucleic acid detection sensor to which the reference electrode is not provided is shown in FIG. 8A, FIG. 8B, FIG. 9A, and FIG. 9B, it is preferable to provide the reference electrode. The reference electrode may be a comb electrode which is mutually engaged to the nucleic acid chain fixed electrode, and can be of combination, float, and be type electrode in each other as shown in FIGS. 9A and 9B.

[0051] An example of the circuit of the embodiment to which the reference electrode is provided in each nucleic acid chain fixed electrode will be described later.

[0052] The outline of the nucleic acid detection system to which the nucleic acid detection sensor according to the present invention is arranged is shown in FIG. 10.

[0053] The nucleic acid detection System 1007 shown in FIG. 10 comprises a nucleic acid detection sensor 1001, a nucleic acid detection sensor fixation device 1002, an electric signal measurement device 1003, a CPU 1004, a power supply 1005, and, a display device 1006.

[0054] The nucleic acid detection sensor is usually attachably and detachably arranged on the substrate 1102 with the connection terminal 1101, and stored in the container 1108 as shown in FIG. 11 in the above-mentioned system. The substrate 1102 has a connection terminal insertion part 1201 in the surroundings thereof, for example, as shown in FIG. 12. In FIG. 11, the test liquid 1103 is injected from the test liquid injection entrance 1105 provided to the bottom to soak the nucleic acid detection sensor 1104 in a state of closing the test liquid outlet 1106. After the nucleic acid detection sensor 1104 is soaked with the test liquid 1103, the nucleic acid included in the test liquid 1103 is hybridized with the nucleic acid chain fixed electrode (not shown in the figure) on the nucleic acid detection sensor 1104. When the nucleic acid detection sensor 1104 is humidified while hybridizing, the evaporated test liquid is exhausted through an air hole 1107. The target nucleic acid is hybridized with the nucleic acid chain fixed electrode (not shown in the figure) on the nucleic acid detection sensor 1104 if the target nucleic acid is included in test liquid. Therefore, after the test liquid 1103 is exhausted from the test liquid outlet 1106, the test liquid 1103 keeps combining with the nucleic acid chain fixed electrode. The test liquid injection entrance 1305 and the test liquid outlet 1306 may be provided at a vertical position of substrate 1302 as shown in FIG. 13.

[0055] Hereinafter, the operation to obtain the finding of the target nucleic acid chain in the test liquid or the

probe nucleic acid chain with a nucleic acid detection sensor according to the present invention will be explained in detail.

[0056] First, the test liquid including the target nucleic acid chain is injected to the space which lies between the nucleic acid chain fixed electrode and the counter electrode.

[0057] The detected target nucleic acid chain is not especially limited, and may be the nucleic acid chain of virus, bacillus, fungous, and helminth etc., a cause genes of an inherited disease, and a marker genes of various diseases etc. For example, it is possible to be used to detect virus infection syndromes such as hepatitis virus (A, B, C, D, E, F, and G type), HIV, influenza virus, herpes group virus, adenovirus, human polyoma virus, human papilloma virus, human parvovirus, mumps virus, human rotavirus, enterovirus, Japanese encephalitis virus, dengue fever virus, rubella viruses, and HTLV, bacillus infection syndromes such as yellow staphylococcus, hemolytic streptococcus, escherichia coli, enteritis vibrio, helicobacter pylori, campylobacter, cholera bacterium, dysentery bacterium, salmonella, senior ell, gonococcus, and squirrel terrier bacterium, leptospire, legionella bacterium, spirochete, pneumonia mycoplasma, rickettsia, chlamydia, malaria, dysentery amoebas, and causes of a disease fungous, and helminth, fungous, parasite or fungus. It can be also used for a check of inherited disease, retina bud cell tumor, Wilm's tumor, family character large intestines polyposis, inherited non-polyposis colic cancer, neurofibroma, familial breast cancer, xeroderma pigmentosum, brain tumor, mouth cancer, gullet cancer, stomach cancer, and colic cancer, check of tumor disease such as liver cancer, pancreatic cancer, lung cancer, goiter, mastadenoma, urinary organs tumor, virilia tumor, female genital tumor, ecphyma, bone-soft part tumor, leukemia, lymphoma, solid tumor, etc. It can be adopted to all fields to which the gene check is necessary in the food check, quarantine, medicine check, legal medicine, agriculture, stock raising, fishery, and forestry, etc. besides the medical treatment. In addition, the detection of restriction fragment length polymorphism (RFLP), single nucleotide polymorphisms (SNPs), and the micro satellite array, etc. is also possible. It is also possible to use for analyzing the unknown the base sequence.

[0058] The test liquid which contains these target nucleic acids is not especially limited, for example, blood, serum, white blood corpuscle, urine, service, semen, saliva, organization, culture cell, and expectoration, etc. may be used. The nucleic acid component is usually extracted from these test liquids. The extraction method is not especially limited, and may use liquid-liquid extraction methods of phenolchloroform method, etc. and the solid-liquid extraction methods which use carrier. It is also possible to use method QIAamp of extracting a nucleic acid on the market (made by the QIAGEN company) and Sumai-test (made by the Sumitomo Metal Industries, Ltd. company), etc.

[0059] The hybridization reaction is performed by the extracted nucleic acid component and the nucleic acid chain detection electrode after the test liquid is injected into the space. A reactive solution is performed in the buffer liquid within the range of ion intensity 0.01 to 5 and the range of pH5 to pH10. Sulfuric acid dextran, salmon sperm DNA, sweetbread DNA, EDTA, and the surface-active agent, etc. which are the hybridization promotion medicine in this solution can be added. The nucleic acid component extracted here is added, and thermal deformed at 90°C or more. The insertion of the nucleic acid chain detection electrode can be performed immediately after deformation or after quick cooling to 0°C. A reactive speed can be improved by the operation such as the stir or shaking while reacting. A reactive temperature is within the range of 10°C to 90°C and a reactive time is from one minute or more to about one evening. The hybridization reaction can be controlled electrochemically and necessary time of reaction can be shortened to a few minutes by applying for the plus potential to the nucleic acid chain fixed electrode, but it is necessary from several hours to several days, conventionally. On the other hand, nonspecific bond can be removed by applying for minus potential to the surface of the electrode.

[0060] When the hybridization reaction ends, the nucleic acid chain fixed electrode is washed. The buffer liquid within the range of pH5 to pH10 and the ion intensity thereof is within the range of 0.01 to 5 is used for washing.

[0061] The duplex chain cognitive body, that is, an intercalator which selectively combines with duplex part (hybrid of the probe nucleic acid chain and the target nucleic acid chain) formed on the surface of the electrode after washing is react, and an electrochemical measurement is performed. The duplex chain cognitive body is a double stranded nucleic acid recognizing substance which binds specifically to a double stranded nucleic acid and is active physicochemically to the reaction system of said nucleic acid probe and said gene sample. The intercalator used here is not limited and bisintercalator, trisintercalator, and polyintercalator, etc. such as Hoechst 33258, acridine orange, quinacrine, daunomycin, metalointercalator, and bisacridine can be used as the intercalator. It is also possible to use the living body high molecules such as organic compounds such as metallic complex such as ruthenium which is called metalointercalator, cobalt, and iron and ethidium bromide, antibodies, and ferments.

[0062] The concentration of the intercalator is different depending on the kind, but it is used within the range of generally 1 ng/mL to 1 mg/mL. In this case, the buffer liquid within the range of pH5 to 10 and the ion intensity within the range of 0.001 to 5 is used.

[0063] The nucleic acid chain fixed electrode is washed after reacting with the intercalator, and an electrochemical measurement is performed. An electrochemical measurement is performed by three electrode

type, that is, the reference electrode, the counter electrode and the action electrode or two electrode type, that is, the counter electrode and the action electrode. In the measurement, a potential more than a potential to which the intercalator electrochemically reacts is applied, and the reactive current value which derives from the intercalator is measured. In this case, the potential can be swept at a fixed velocity, or be applied with the pulse or can be applied with fixed potential. The current and the voltage are controlled by using devices such as potentiostat, digital multi meters, and the function generators for the measurement. The concentration of the target gene is calculated from calibration curve based on the obtained current value.

[0064] An electrochemical signal can have the oxidation reduction current change, the oxidation reduction potential change, the electric capacity change, the resist change, and the electrochemistry emission change to the index. The effect of these signal change is promoted by using the material which combines with duplex chain nucleic acid such as intercalator etc. specifically.

[0065] The first nucleic acid detection sensor according to the present invention is characterized by arranging the nucleic acid chain fixed electrode and the counter electrode oppositely so that the test liquid may flow between the nucleic acid chain fixed electrode and the counter electrode.

[0066] FIG. 14C and FIG. 14D show the nucleic acid detection sensors which construct the conventional DNA array. A plurality of nucleic acid chain fixed electrodes 1401, to which the probe nucleic acid chain 1402 with an already-known array is fixed, and the counter electrode 1404 are arranged on the same substrate 1403, and the test liquid 1406 flows on above-mentioned substrate 1403. In the corresponding arrangement, the distance of the counter electrode 1404 and each nucleic acid chain fixed electrode 1402 is different in each nucleic acid fixed electrode 1401. In such a configuration, there is a case the distance of the nucleic acid chain fixed electrode 1401 and the counter electrode 1404 might become far as shown in the left end of the figure, and the response speed becomes slow. Since the distance of the counter electrode 1404 and each nucleic acid chain fixed electrode 1401 is different in each nucleic acid chain fixed electrode 1401, enough measurement accuracy cannot be achieved.

[0067] On the other hand, in the first nucleic acid detection sensor according to the present invention shown in FIG. 14A and FIG. 14B, the nucleic acid chain fixed electrode 1401 and the counter electrode 1404, to which the probe nucleic acid chain 1402 is fixed, are plate-like electrodes, and are arranged oppositely to grasp so that the test liquid. According to the corresponding arrangement, all of each nucleic acid chain fixed electrode 1401 on the first substrate 1403 can be arranged to an equal distance from the counter electrode 1404 on the second substrate 1405 and the neighborhood of the counter electrode 1404. Therefore, by

using the nucleic acid detection sensor in which the electrodes are arranged in such a manner, it becomes possible to apply an equal voltage to all the target nucleic acids in the test liquid 1406, which is hybridized with the probe nucleic acid chain 1402 on each nucleic acid chain fixed electrode 1401, to be detected. Therefore, the measurement accuracy and the response speed improve. Since the test liquid 1306 is injected between the first substrate 1303 and the second substrates 1305, the amount of a necessary test liquid can be decreased. FIG. 14A shows the nucleic acid detection sensor that the reference electrode 1407 is not arranged on the first substrate 1403. FIG. 14B shows the nucleic acid detection sensor that the reference electrode 1407 is arranged on the first substrate 1403.

[0068] In the first nucleic acid detection sensor according to the present invention, when the nucleic acid chain fixed electrode is formed on the insulation substrate, the counter electrode is arranged to place a flow path of the test liquid with the nucleic acid chain fixed electrode, and is arranged in a substrate different from the substrate where the nucleic acid chain fixed electrode.

[0069] The position of the counter electrode and the nucleic acid chain fixed electrode is not especially limited. It is desirable to arrange the counter electrode to become an equal distance from all the nucleic acid chain fixed electrodes to apply an equal electrode to all the nucleic acid chain fixed electrodes, though both electrodes are arranged on a different substrate. For example, when the nucleic acid chain fixed electrode is arranged on a plane, the counter electrode may be arranged on a plane parallel to the plane. When the nucleic acid chain fixed electrode is arranged on the sphere, the counter electrode may be arranged on the concentric sphere of the sphere.

[0070] In the first nucleic acid detection sensor, it is preferable the both of the nucleic acid fixed electrode and the counter electrode have flat surfaces and the flat surfaces are arranged oppositely.

[0071] The first nucleic acid detection sensor according to the present invention comprises a plurality of nucleic acid detection cells, and one or more nucleic acid chain fixed electrodes is arranged in each cell. The counter electrode may be provided for each nucleic acid chain fixed electrode. The counter electrode may be common among the plurality of nucleic acid detection cells and may be one, for example, in a word, for the plurality of nucleic acid chain fixed electrodes.

[0072] The material of the counter electrode which should be arranged in the first nucleic acid detection sensor according to the present invention and the distance with the nucleic acid chain fixed electrode are the above-mentioned.

[0073] It is general to arrange the reference electrode in the same substrate as the nucleic acid fixed electrode when the reference electrode is arranged in the first nucleic acid detection sensor according to the present in-

vention. The reference electrode may be arranged in the part excluding this.

[0074] The second nucleic acid detection sensor according to the present invention is characterized by comprising a reference electrode in each cell.

[0075] In the second nucleic acid detection sensor according to the present invention, the counter electrode may be common to the plurality of nucleic acid chain fixed electrode or may be arranged for each nucleic acid detection cell. The counter electrode may be connected with either of the signal line or scanning line when the plural connect counter electrodes are arranged.

[0076] The material of the nucleic acid chain fixed electrode, the counter electrode, and the reference electrode and the operation etc. for the measurement of nucleic acids are as being described to a general configuration of the nucleic acid detection sensor according to the present invention mentioned above and directions. That is, by using the electrochemical reaction by the hybrid nucleic acid chain formed between the probe nucleic acid chain and the target nucleic acid chain, whether or not the probe nucleic acid chain or the target nucleic acid chain has a specific base sequence is detected.

[0077] Thus, the measurement accuracy improves by decreasing the uncompensatory resistance between the nucleic acid chain fixed electrode and the reference electrode if the reference electrode is provided to each nucleic acid chain fixed electrode. It is preferable to comprise the reference electrode to each nucleic acid chain fixed electrode to control the potential of each nucleic acid chain fixed electrode.

[0078] The second nucleic acid detection sensor according to the present invention uses, for example, the potentiostat circuit for the minute current measurement comprising an operational amplifier 1607 which functions as a control amplifier, a voltage follower amplifier, and a current follower amplifier, an operational amplifier 1608, and an operational amplifier 1609 as shown in FIG. 15. The plurality of nucleic acid chain fixed electrodes are actually arranged to the second nucleic acid detection sensor according to the present invention though only one nucleic acid chain fixed electrode is shown in the potentiostat circuit of FIG. 15 for easiness.

[0079] This circuit comprises three operational amplifiers with the function of each control amplifier, voltage follower amplifier, and the current follower amplifier. These circuits are different from the conventional circuit in the point for the minute current measurement. Therefore, the potentiostat circuit used in the nucleic acid detection sensor may be a circuit for the minute current measurement.

[0080] The function of each operational amplifier in the circuit of FIG. 15 is as follows.

[0081] The operational amplifier 1607 configures a part of the inversion amplifier, and by applying the voltage of $(1+Zf/Rf)$ times of e_f (e_f is assumed to mean the potential of point f when the common potential is as-

sumed to be reference here, and it is same as follows) to the counter electrode 1602. e_f is kept constant for e_a (That is, V_{cc}) (Here, Z_f shows electrochemical impedance from counter electrode 1602 to the reference electrode 1603). Since the operational amplifier has the negative feedback, e_a is equal to e_b (potential of common). In the figure, though the common is grounded, it is not unnecessary to ground.

[0082] The operational amplifier 1608 has a function to amplify an input power to Z_{in}/Z_{out} times (Z_{in} and Z_{out} are the input impedance and output impedances, respectively). Since Z_{in} is very high compared with Z_{out} , the output power becomes remarkably large compared with the input power. Internal resistance of the reference electrode 1603 can be disregarded by the function of the operational amplifier 1608.

[0083] Since the operational amplifier 1609 has the negative feedback, e_g is equal to e_h , therefore, when the nucleic acid chain fixed electrode 1601 is connected with the signal with the switching element 1604, the potential of the nucleic acid chain fixed electrode 1601 becomes equal with the potential of common. Therefore, the operational amplifier 1609 operates to keep the potential of the nucleic acid chain fixed electrode 1601 which is the action electrode to the common potential. If the ratio of the resistance (not shown in the figure) between point 0 and point a and the resistance between point a and point f is set to 1 when the input voltage is assumed to be V , the potential of the reference electrode becomes $-V$ by the action of the operational amplifier 1603. The resistance of resistor in the circuit and the presence of the use of the resistance may be properly selected according to the desired amplification rate etc. Since the potential of the nucleic acid chain fixed electrode 1601 is equal to the potential of common, an equal voltage to the input voltage is accurately applied between the nucleic acid chain fixed electrode 1601 (action electrode) and the reference electrode 1603. The current caused by applying the voltage to the nucleic acid chain fixed electrode 1601 by the switching element 1604 connected with the scanning line 1606 from point g on signal line 1605 to point i through the resistor 1610 since the point g is grounded virtually. The largeness of the current can be measured by measuring the voltage down by the resistor 1610.

[0084] When the resistor 1610 is arranged between a point g and a point i, the error is occurred in the potential of the nucleic acid chain fixed electrode 1601 by the potential difference at both ends of the resistor. However, even if the resistor 1610 is arranged between the point g and the point i, the error is not occurred in the potential of the nucleic acid chain fixed electrode 1601 since e_g is kept the potential of common. Therefore, it becomes possible to measure an electrochemical measurement of high accuracy.

[0085] The circuit of FIG. 16 is another potentiostat circuit used for the second nucleic acid detection sensor, and has a function to keep the voltage to be constant as

well as the circuit of FIG. 15. Therefore, the functions of operational amplifiers 1707, 1708 and 1709 are the same as the corresponding operational amplifiers shown in FIG. 15.

5 [0086] Since the reference electrode is arranged to each nucleic acid chain fixed electrode as well as FIG. 15, the sensor circuit for the nucleic acid detection of this embodiment has the measurement accuracy in the sensor for compared with the conventional circuit.

10 [0087] Though only one reference electrode is drawn in FIG. 16 for easiness, one or more reference electrodes are arranged to each nucleic acid chain fixed electrodes, actually.

15 [0088] Since the reference potential is held concurrently in the circuit of FIG. 16 by the potential applied to scanning line, the wiring which is led from non-inversion input terminal of the operational amplifier 1708 is used together to the plurality of electrode, and not included in the number of wirings for the nucleic acid detection cell.

20 [0089] As mentioned above, the nucleic acid detection sensor of this embodiment can achieve very high measurement sensitivity with simple wiring.

[0090] The circuit of FIG. 17 is still another potentiostat circuit used for the nucleic acid detection sensor of the second embodiment, and the circuit of FIG. 17 has the function to keep the voltage to be constant as well as the circuits of FIG. 15 and FIG. 16. Therefore, details of the function of potentiostats 1807, 1808 and 1809 are as described in FIG. 15 or FIG. 16.

30 [0091] The circuit of FIG. 17 is different from the circuit of FIG. 16, and the reference electrode 1803 is connected with not the scanning line 1806 but the signal line 1804. Therefore, the reference electrode 1803 is not connected with the scanning line 1806 in the circuit of FIG. 17. Therefore, the reference potential of the reference electrode does not hold concurrently with the potential of the scanning line 1806, and the applied potential can be freely set. Therefore, many kinds of intercalators can be used compared with the circuit of FIG. 16 in the circuit of FIG. 17.

[0092] The switching element may be omitted though the reference electrode 1803 is connected with switching element 1804 in FIG. 17.

45 [0093] The nucleic acid chain fixed electrode 1801 and the reference electrode 1803 are placed both sides of the lead which are connected with the non-inversion input terminal of the operational amplifier 1808 in FIG. 17. The reference electrode 1803 may be arranged on the same side as the nucleic acid chain fixed electrode 1801 so that two electrodes are faced.

50 [0094] As mentioned above, the circuit of FIG. 17 can achieve high measurement sensitivity, and can use many kinds of intercalators.

55 [0095] The third nucleic acid detection sensor according to the present invention will be explained referring to FIG. 18 to FIG. 21. The third nucleic acid detection sensor according to the present invention is characterized in that the signal lines are shared by the switching

element.

[0096] FIG. 18 is upper figure of the nucleic acid detection sensor which is usually used. In FIG. 18, the nucleic acid chain fixed electrodes 1901 to which the probe nucleic acid chain (not shown in the figure) is fixed are arranged in the X-Y matrix of 4×3 . The counter electrode is omitted in FIG. 18, though the counter electrode is located above the plane where the nucleic acid chain fixed electrode 1901 is arranged perpendicular thereto in the sensor for actual nucleic acid detection.

[0097] Each nucleic acid chain fixed electrode 1901 forms the nucleic acid detection cell with the counter electrode.

[0098] Each nucleic acid chain fixed electrode 1901 is connected with the signal line 1903 through the switching element 1902 such as transistors. The signal line 1903 is further connected with the amplifier 1904 and the A/D converter 1905 to amplify the current from the nucleic acid chain fixed electrode 1901.

[0099] Since the clock signal is output from the timing pulse generator 1909 to the switching element 1902 through the scanning line 1906, the nucleic acid chain fixed electrodes 1901 are scanned to become active from the left end to an arrow direction in the figure one by one. The counter 1908 and the X decoder 1907 of FIG. 18 control the ON-OFF on the signal line. When the nucleic acid chain fixed electrode 1901 becomes active, the voltage is applied between the nucleic acid chain fixed electrode 1901 and the counter electrode (not shown in the figure) and the intercalator inserted in the target nucleic acid which hybridizes with the nucleic acid chain fixed electrode 1901 is oxidized. After an electric change occurred at oxidation is amplified with said the amplifier 1904 through the signal line 1903, an electric change is A/D-converted with the A/D converter 1905.

[0100] FIG. 19 is a figure, which shows an example of the circuit of the third nucleic acid detection sensor according to the present invention. In the nucleic acid detection sensor of FIG. 19, the point, in which the switching element is also arranged in a row direction and the scanning is performed from upper side to lower side of the FIG. 19, is different from the nucleic acid detection sensor of FIG. 18.

[0101] In FIG. 19, the nucleic acid chain fixed electrode 2001 is arranged in the X-Y matrix of 4×3 , and the nucleic acid chain fixed electrode 2001 and the counter electrode (not shown in the figure) construct the nucleic acid detection cell.

[0102] Each nucleic acid chain fixed electrode 2001 is connected with the signal line 2004 through the amplifier 2002 and the electrode switching element 2003. The signal line switching element 2005 is connected with a part of each signal line 2004, and thereafter the signal lines 2004 become one line, and is connected with the A/D converter 2006.

[0103] An electric signal is output from the column direction scanning circuit, which is constructed by the X

decoder 2007 and the counter 2008, to the electrode switching element 2003 one by one through the signal line 2012. On the other hand, an electric signal is output from the row direction scanning circuit, which is constructed by the Y decoder 2009 and the counter 2010 to the signal line switching element 2005 one by one.

[0104] If the clock signal generated from the timing pulse generator 2011 is output to the column direction scanning circuit and the row direction scanning circuit respectively as X direction clock signal and Y direction clock signal as shown in FIG. 20, the voltage is applied to the electrode from the first column and first row line (electrode in the top of the left), the first column and second row line, the first column and third row line, and the second column and first row line ... one by one. An electric change, which takes place because of the application for the voltage is measured as a serial signal, and is A/D-converted into the output signal with the A/D converter.

[0105] In the nucleic acid detection sensor of FIG. 19, to detect an row direction electric signal one by one, the nucleic acid detection sensor using the scanning circuit, which is constructed by the decoder and the counter is shown. The decoder and the counter of FIG. 19 can be replaced with the shift register circuit 2210 as shown in FIG. 21. The configuration of the nucleic acid detection sensor of FIG. 21 is the same as the nucleic acid detection sensor of FIG. 19 excluding decoder and the counter being replaced with the shift register circuit. Thus, if the shift register circuit is used, the external circuit configuration becomes simple.

[0106] The third nucleic acid detection sensor shown in FIG. 19 and FIG. 21 has the effect of speeding up the measurement in compared with the nucleic acid detection sensor shown in FIG. 18.

[0107] It can be also possible to use the first to third nucleic acid detection sensors according to the present invention alone, and can be used by properly combining.

Claims

1. A nucleic acid detection sensor **characterized by** comprising:

a plurality of nucleic acid chain fixed electrodes (1401) to which a probe nucleic acid chain is fixed; and

a counter electrode (1404) which is arranged opposite to the nucleic acid chain fixed electrode, wherein a current flowing between the counter electrode and the nucleic acid chain fixed electrode.

2. The nucleic acid detection sensor according to claim 1, **characterized in that** the counter electrode is commonly provided to a plurality number of the nucleic acid chain fixed electrodes.

3. The nucleic acid detection sensor according to claim 1, **characterized in that** the counter electrode is provided for each of the nucleic acid chain fixed electrodes.

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4. The nucleic acid detection sensor according to claim 1, **characterized in that**

each of the nucleic acid chain fixed electrodes has a flat plane to which the probe nucleic acid is fixed,
the counter electrode has a flat plane, and
the flat plane of one of the nucleic acid chain fixed electrodes is arranged to face the flat plane of the counter electrode.

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5. The nucleic acid detection sensor according to claim 1, **characterized in that**

the nucleic acid chain fixed electrodes and the counter electrode is arranged to flow a test liquid therebetween.

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6. The nucleic acid detection sensor according to claim 1, **characterized in that**

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the nucleic acid chain fixed electrodes and the counter electrode are exposed to a test liquid and detect a current change between the nucleic acid chain fixed electrodes and the counter electrode caused by a hybridization of the probe nuclei acid and a nuclei acid in the test liquid.

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7. The nucleic acid detection sensor according to claim 1, **characterized in that**

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a duplex chain cognitive body is added to the test liquid, and
a current change between the nucleic acid chain fixed electrodes and the counter electrode is caused by the duplex chain cognitive body.

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8. The nucleic acid detection sensor according to claim 1, **characterized in that**

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the nucleic acid chain fixed electrodes and the counter electrode are comb electrodes, and arranged to be mutually engaged.

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9. A nucleic acid detection sensor **characterized by** comprising:

a plurality of nucleic acid chain fixed electrodes to which the probe nucleic acid chain is fixed;
a counter electrode, a current flowing between each of the nucleic acid chain fixed electrodes

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and the counter electrode; and
a reference electrode provided for each of the nucleic acid chain fixed electrodes, configured to make a voltage between the nucleic acid chain fixed electrode and the counter electrode constant.

10. The nucleic acid detection sensor according to claim 9, **characterized in that**

the nucleic acid chain fixed electrodes and the reference electrode are comb electrodes and are arranged to be engaged.

11. The nucleic acid detection sensor according to claim 9, **characterized by** further comprising:

a first amplifier which inputs a signal from the reference electrode or a scanning line;
a second amplifier to input a reference potential to apply a predetermined potential to the counter electrode; and
a reference resistor connected between an output side of the first amplifier and the reference potential.

12. The nucleic acid detection sensor according to claim 9, **characterized in that**

the nucleic acid chain fixed electrodes and the counter electrode are exposed to a test liquid and detect a current change between the nucleic acid chain fixed electrodes and the counter electrode caused by a hybridization of the probe nuclei acid and a nuclei acid in the test liquid.

13. The nucleic acid detection sensor according to claim 12, **characterized in that**

a duplex chain cognitive body is added to the test liquid, and
a current change between the nucleic acid chain fixed electrodes and the counter electrode is caused by the duplex chain cognitive body.

14. A nucleic acid detection sensor **characterized by** comprising:

a plurality of nucleic acid chain fixed electrode (2001), to which a probe nucleic acid chain is fixed, arranged in a matrix;
a plurality of scanning lines (2012) configured to select the plurality of nucleic acid chain fixed electrodes one by one;
a plurality of signal lines (2004) configured to transmit a measurement signal from the plural-

ity of nucleic acid chain fixed electrodes;
a plurality of switching elements (2005) connected with the plurality of signal lines; and
an A/D converter (2006) connected with the plurality of switching elements.

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15. The nucleic acid detection sensor according to claim 1 or claim 14, **characterized by** further comprising

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a reference electrode provided for each of the nucleic acid chain fixed electrodes, configured to make a voltage between the nucleic acid chain fixed electrodes and the counter electrode constant.

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16. The nucleic acid detection sensor according to claim 9 or claim 14, **characterized in that**

the counter electrode and the nucleic acid chain fixed electrode are formed on a same plane and the counter electrode is formed so as to surround the nucleic acid chain fixed electrode.

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17. The nucleic acid detection sensor according to claim 14, **characterized in that**

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the nucleic acid chain fixed electrodes and the counter electrode are exposed to a test liquid and detect a current change between the nucleic acid chain fixed electrodes and the counter electrode caused by a hybridization of the probe nucleic acid and a nucleic acid in the test liquid.

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18. The nucleic acid detection sensor according to claim 17, **characterized in that**

a duplex chain cognitive body is added to the test liquid, and
a current change between the nucleic acid chain fixed electrodes and the counter electrode is caused by the duplex chain cognitive body.

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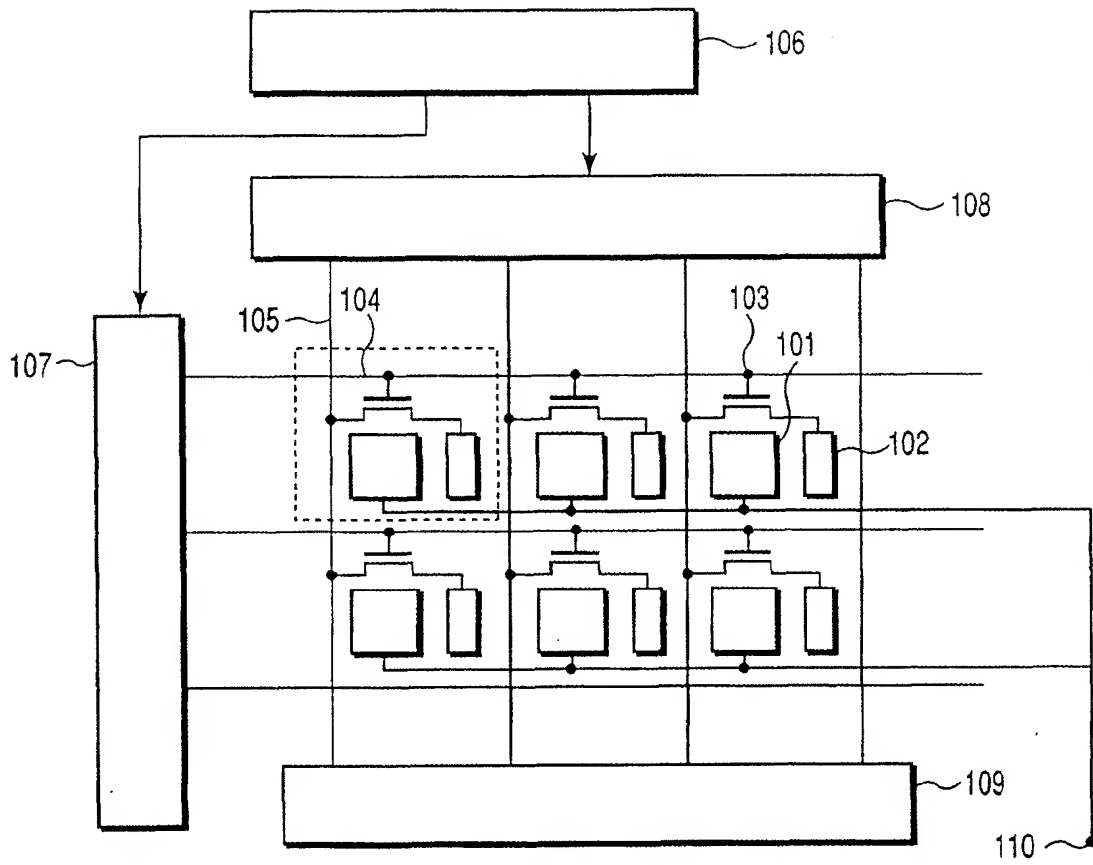


FIG. 1

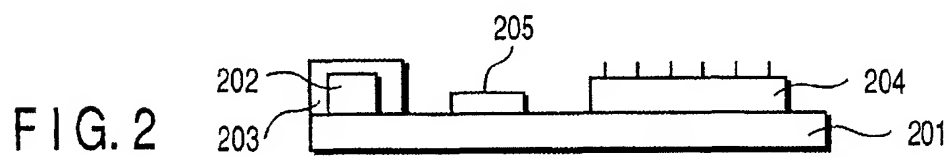


FIG. 2

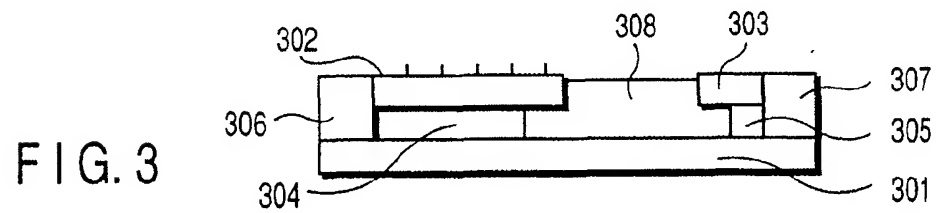
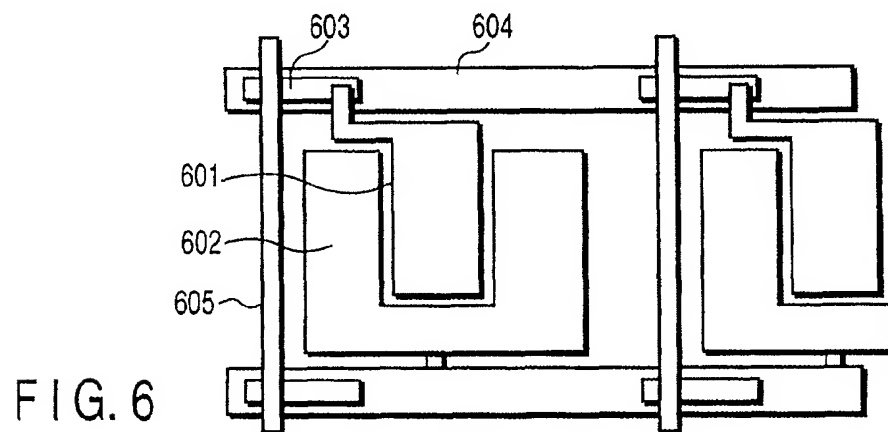
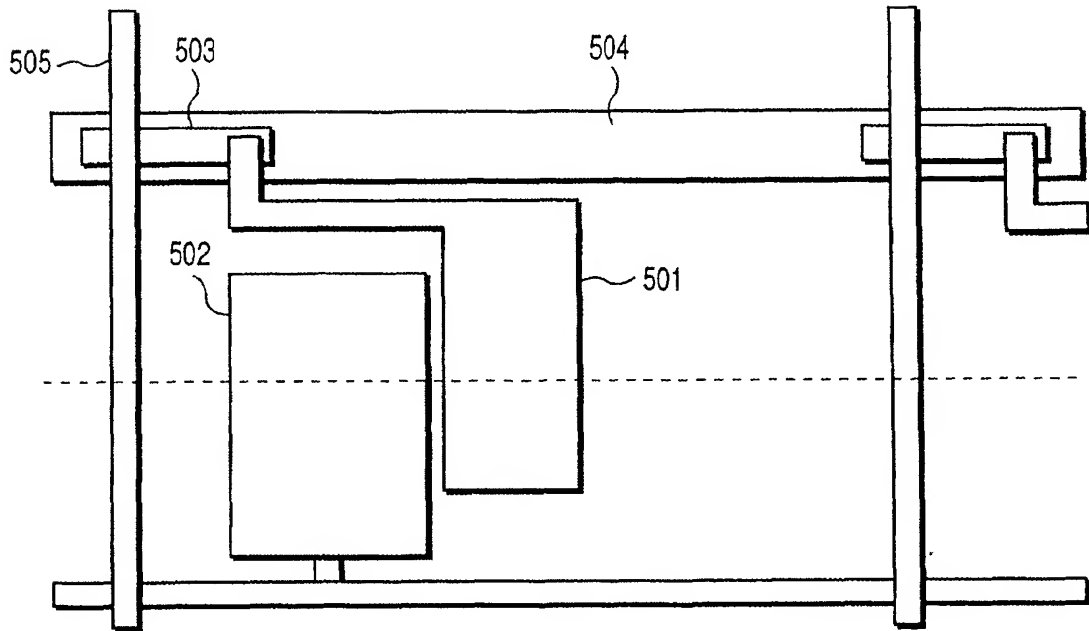
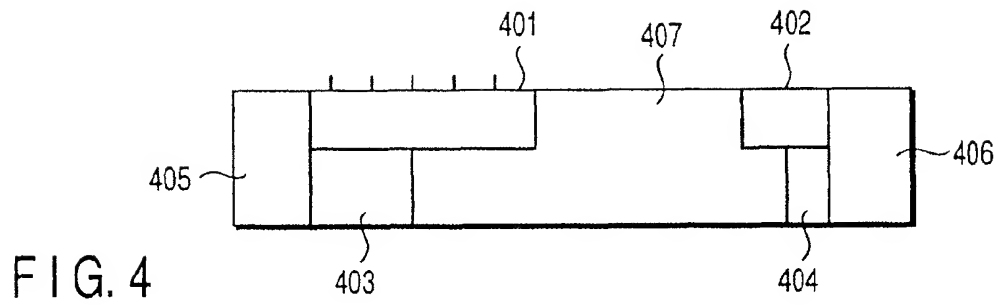


FIG. 3



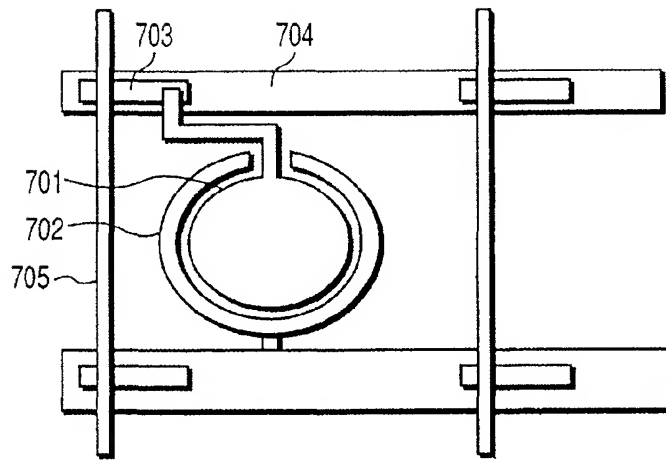


FIG. 7

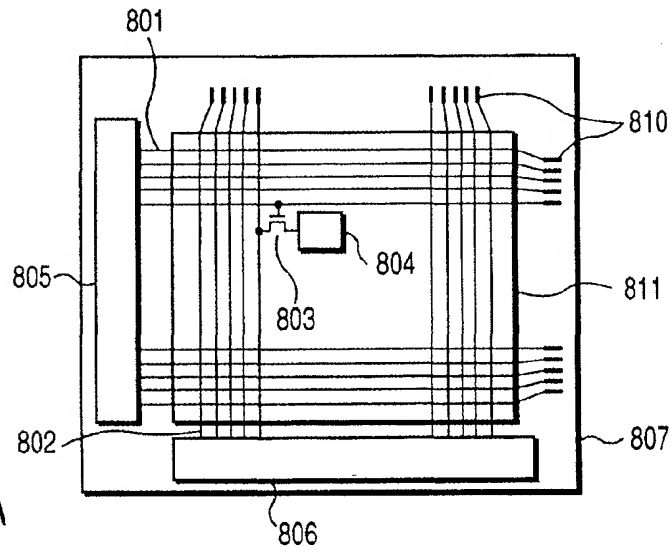


FIG. 8A

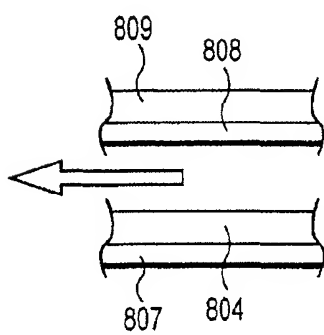


FIG. 8B

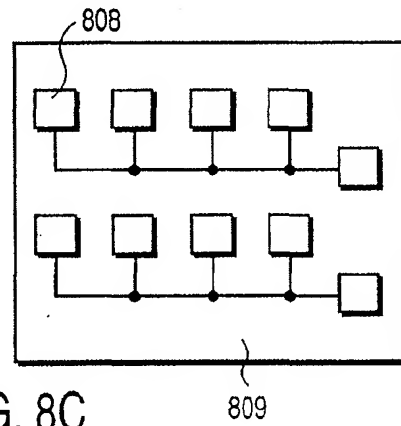
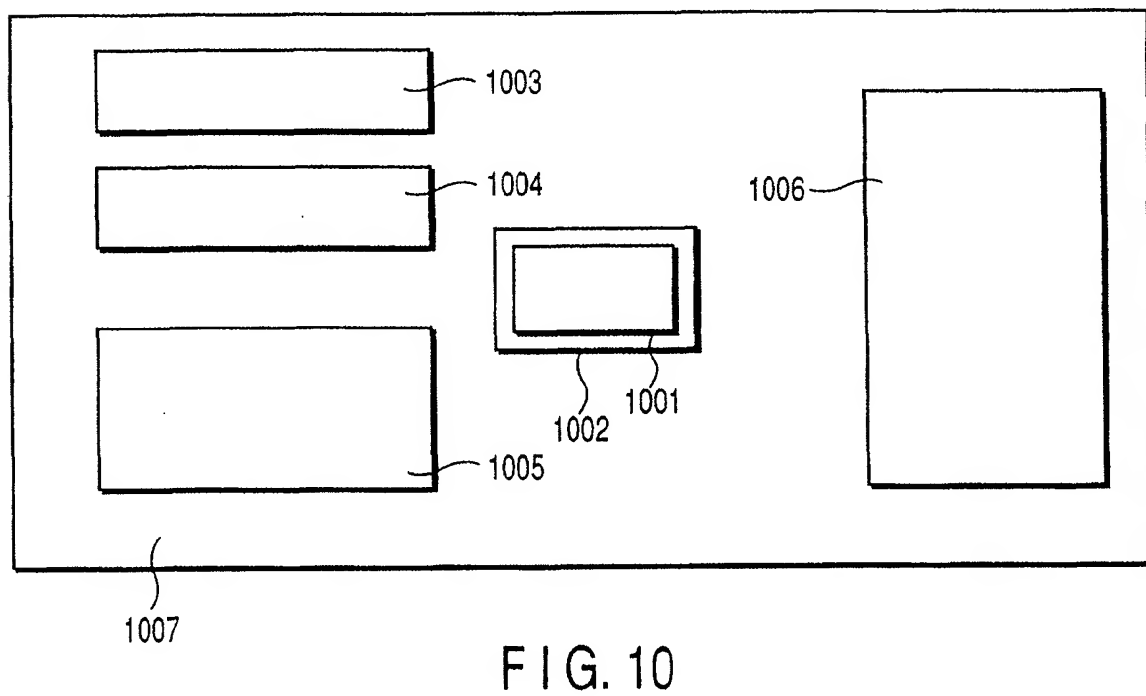
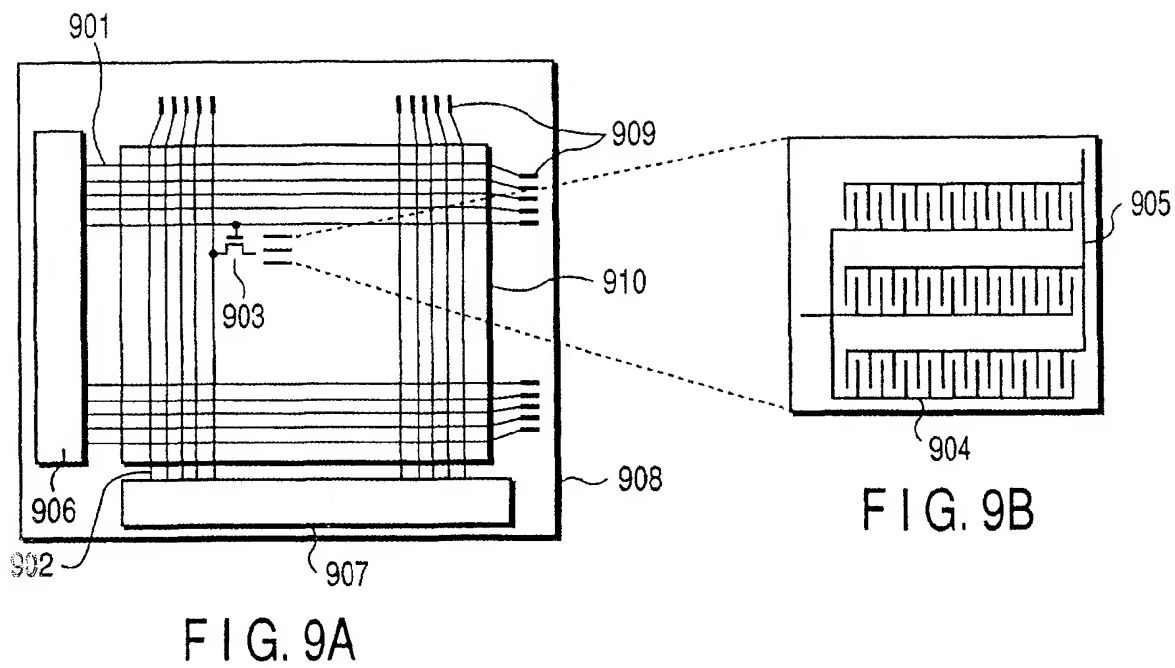
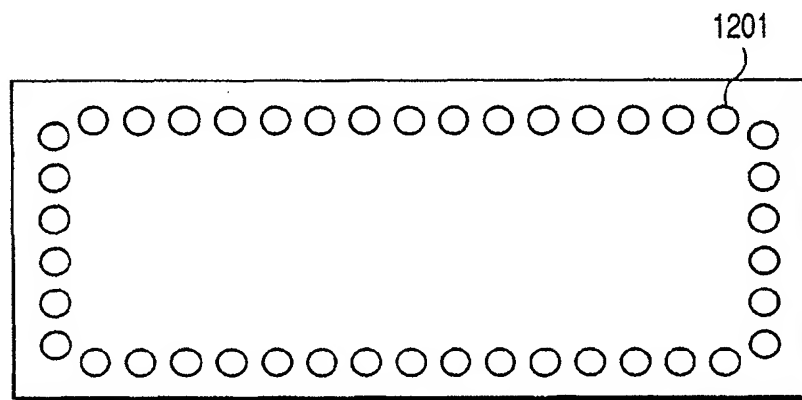
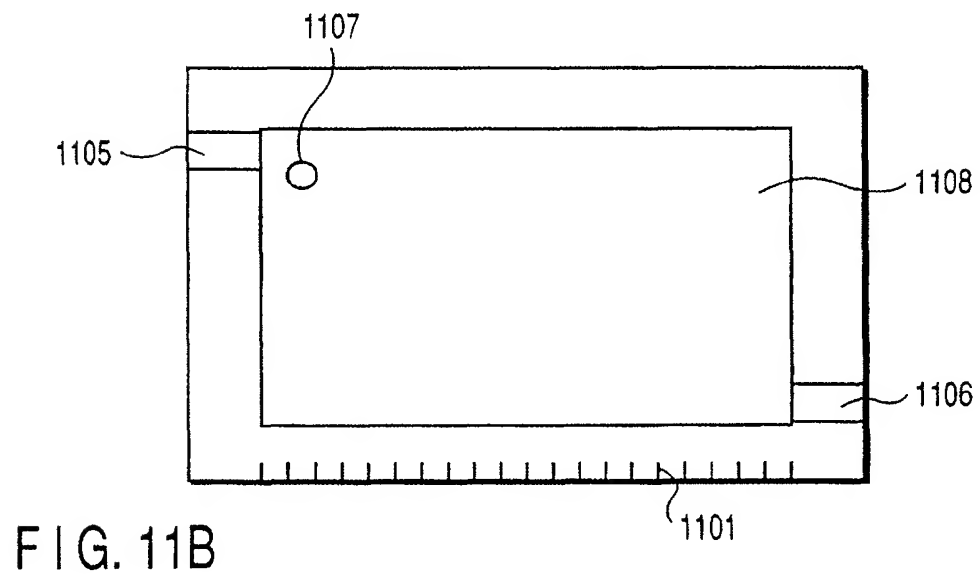
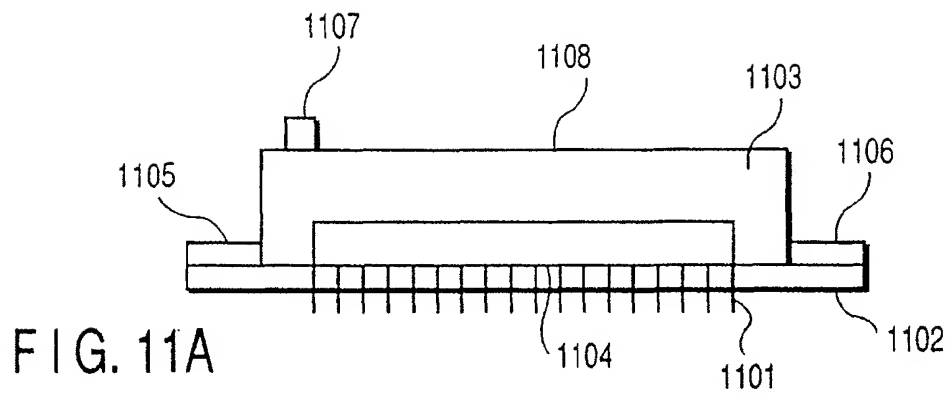


FIG. 8C





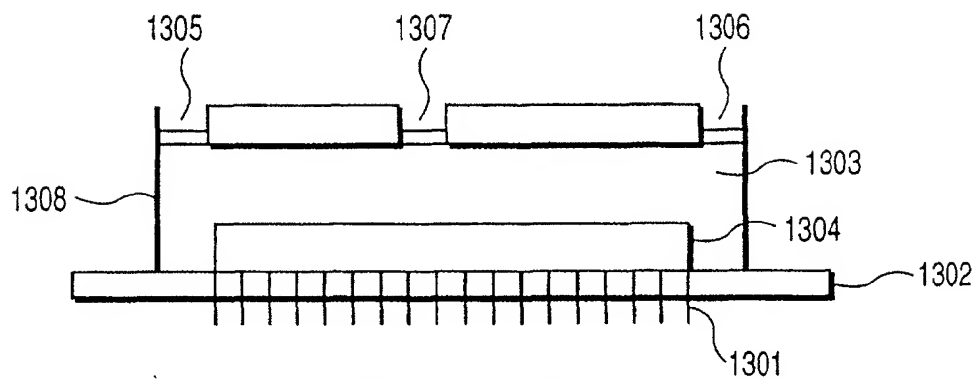


FIG. 13A

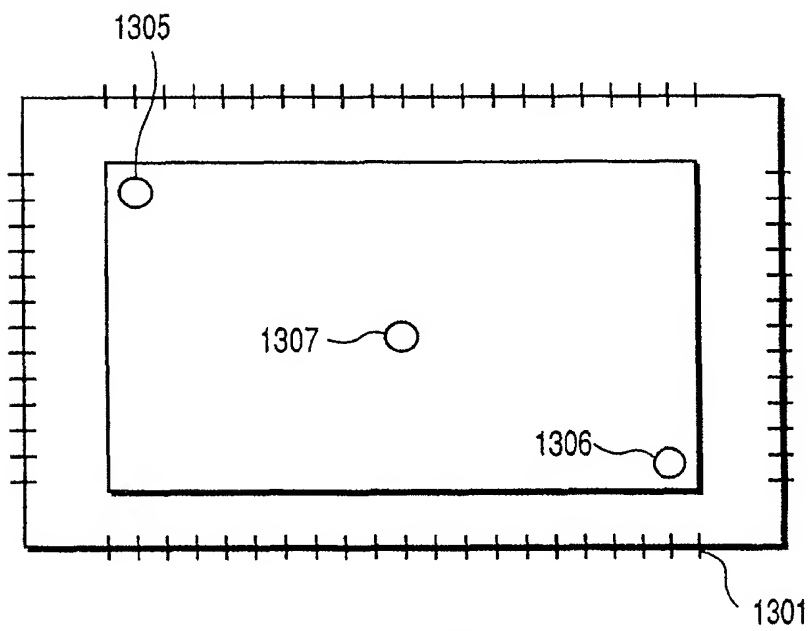
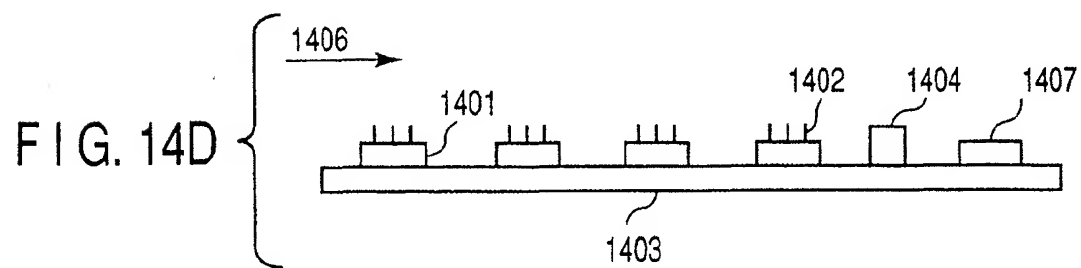
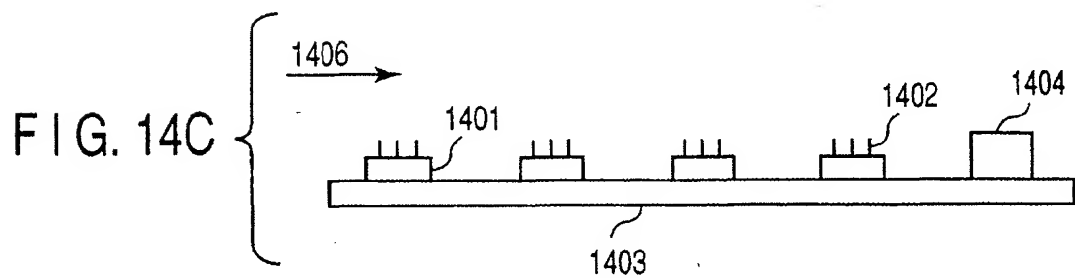
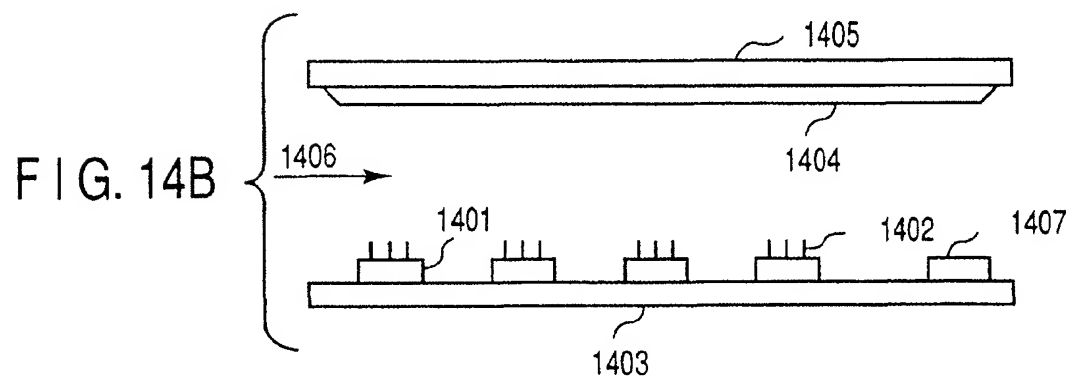
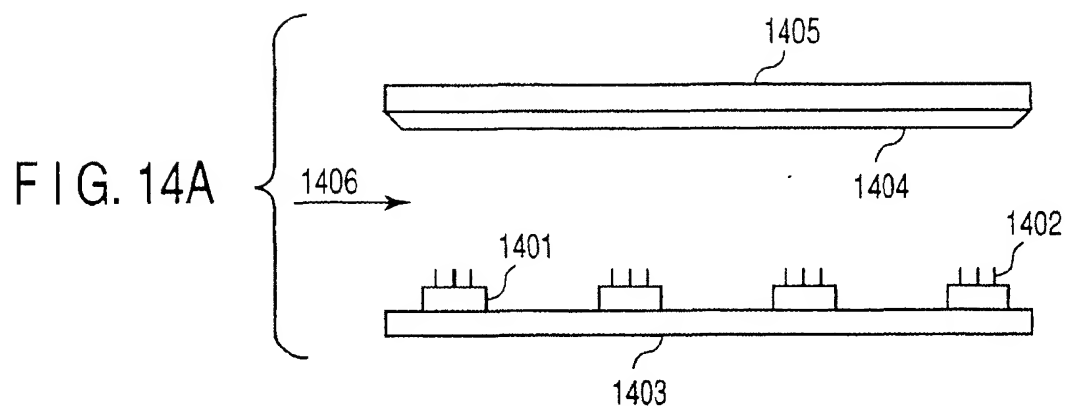
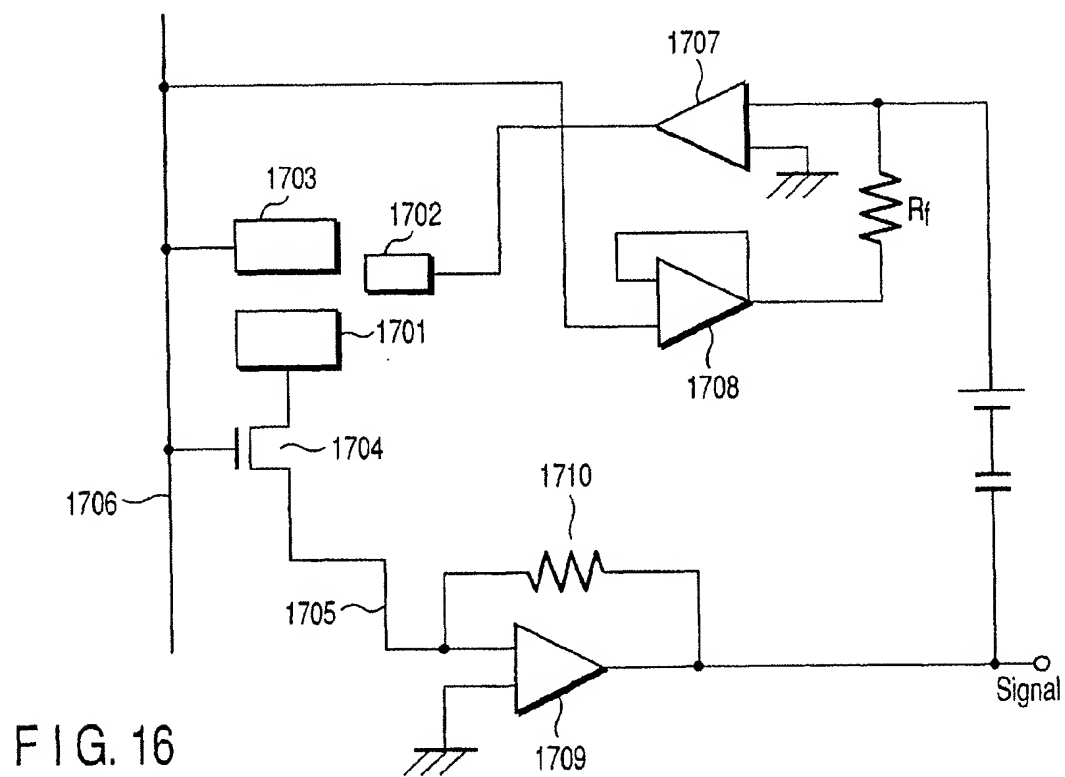
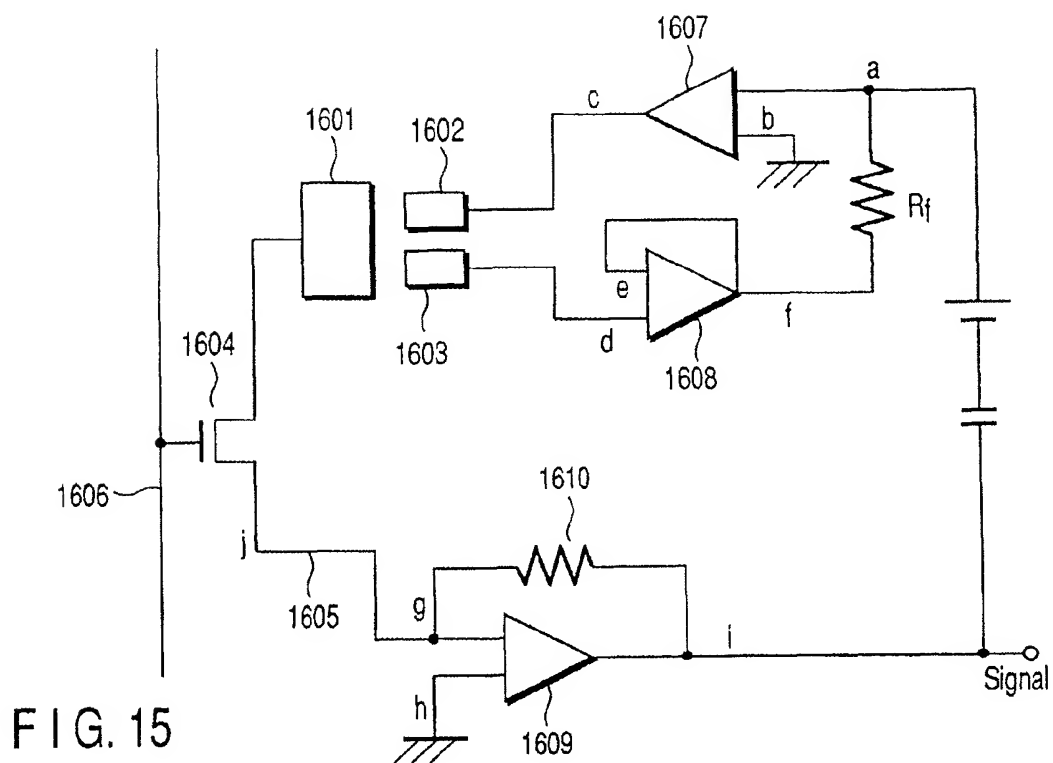


FIG. 13B





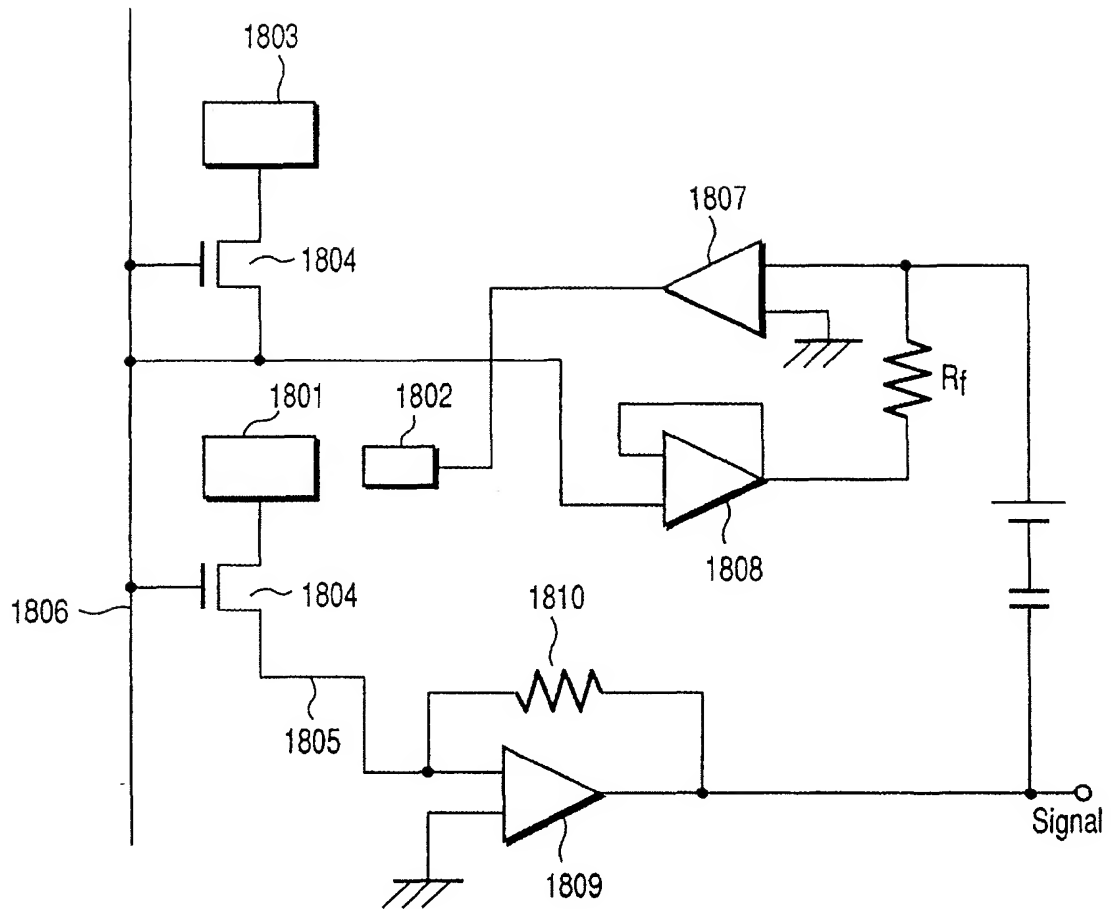


FIG. 17

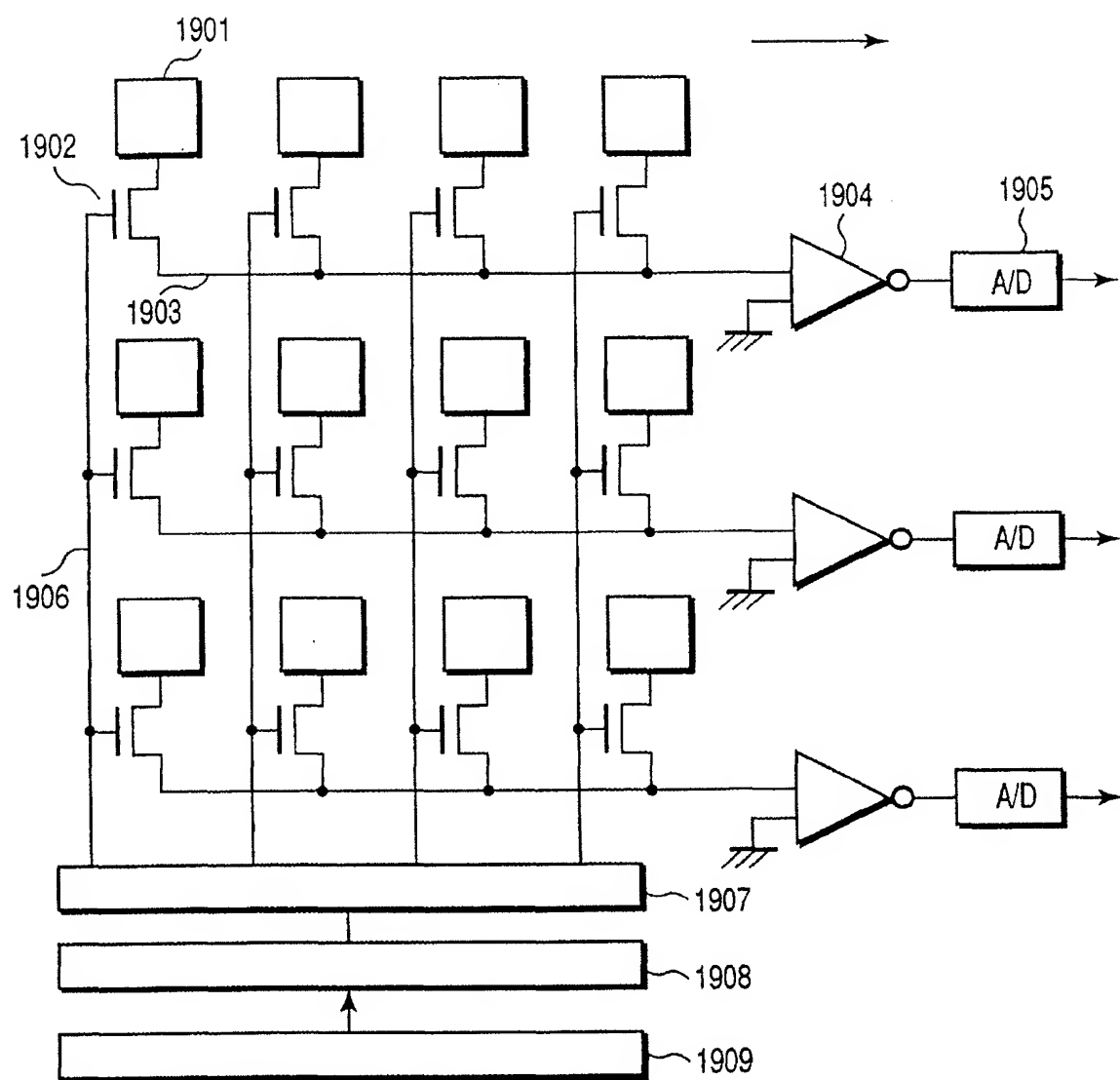


FIG. 18

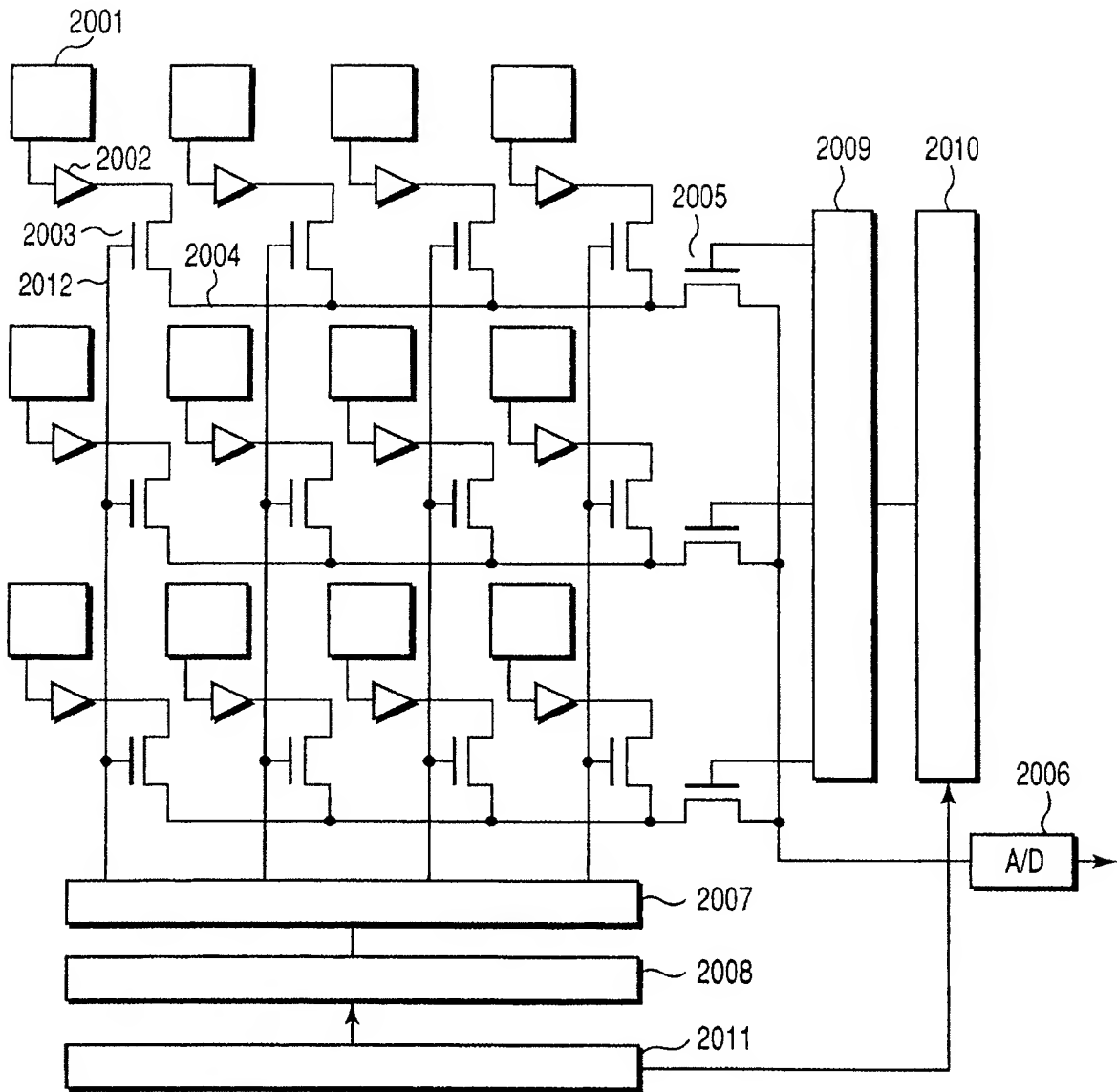


FIG. 19

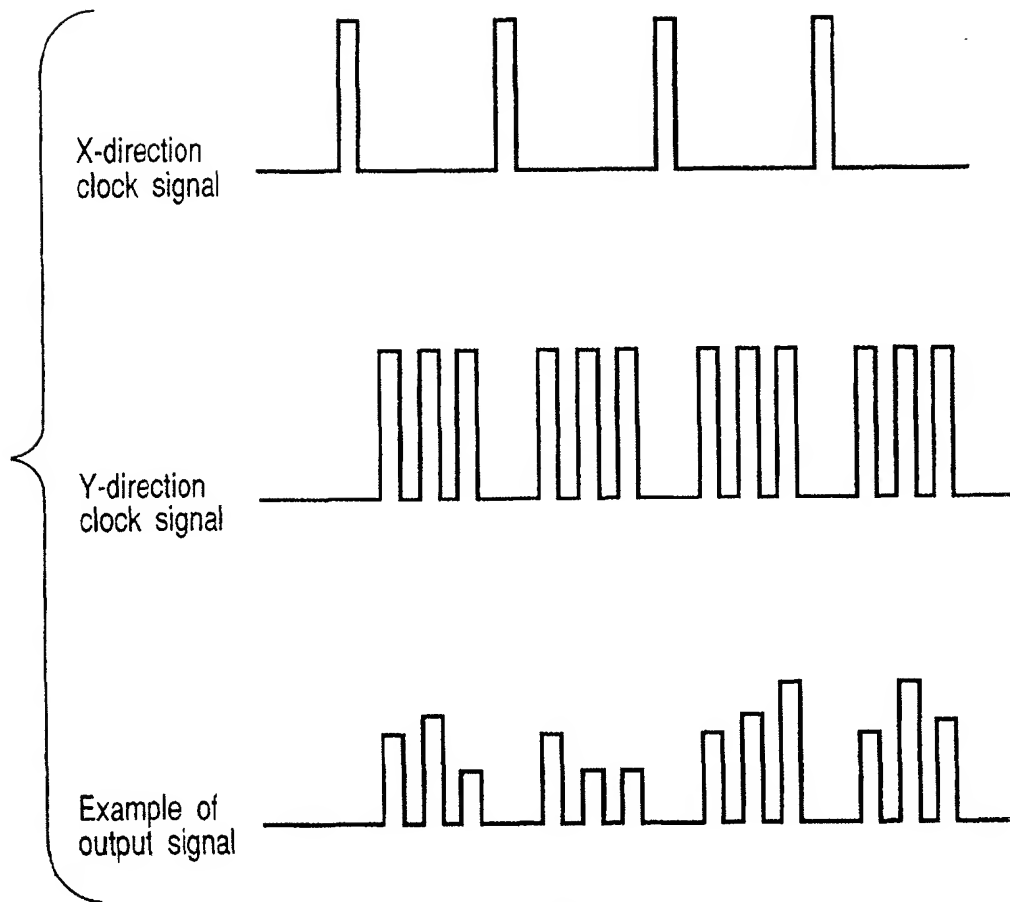


FIG. 20

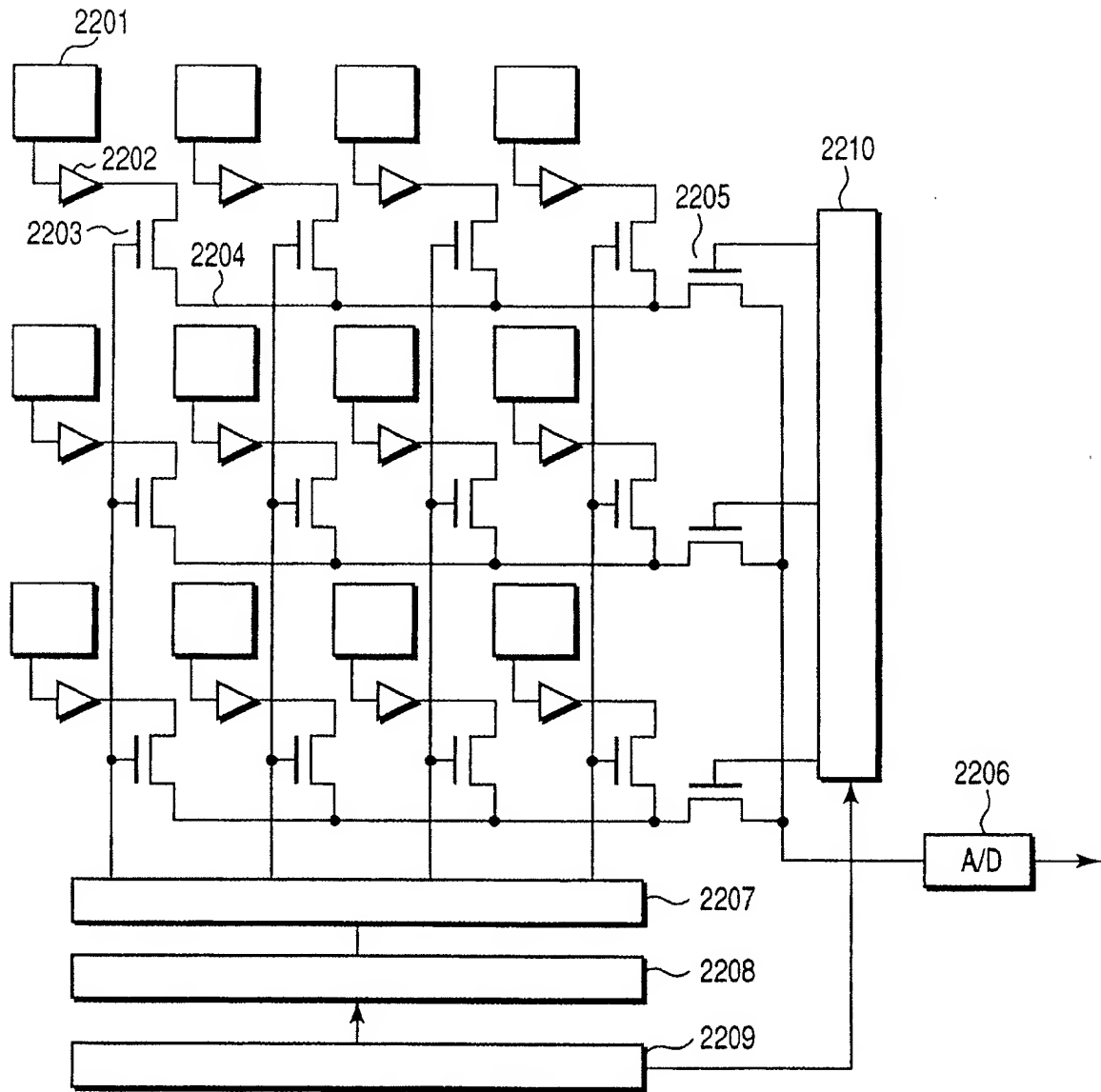


FIG. 21



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 01 30 8139

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	EP 0 798 561 A (MOCHIDA PHARM CO LTD) 1 October 1997 (1997-10-01) * column 8, line 6 - column 9, line 8; figure 1 *	1-8	G01N27/403 C12Q1/68 G01N33/543
X	WO 99 67628 A (LUMLEY WOODYEAR THIERRY DE ;HELLER ADAM (US); HELLER E & CO (US);) 29 December 1999 (1999-12-29) * page 9, line 11 - page 15, line 21; figure 1 *	1-8	
Y	WO 99 27355 A (MIRSKY VLADIMIR M ;RIEPL MICHAEL (DE); WOLFBEIS OTTO SAMUEL (DE)) 3 June 1999 (1999-06-03) * page 5, line 13 - page 8, line 7; figure 1 *	1-8	
Y	US 5 945 286 A (KRIHAK MICHAEL ET AL) 31 August 1999 (1999-08-31) * column 1, line 34 - line 51; figures 1,2 *	1-8	
Y	WO 99 51778 A (BARTON JACQUELINE ;HILL MICHAEL (US); KELLEY SHANA (US); CALIFORNI) 14 October 1999 (1999-10-14) * page 8, line 25 - line 31; figure 1 *	1-8	C12Q G01N
A	WANG J ET AL: "Nucleic-acid immobilization, recognition and detection at chronopotentiometric DNA chips" BIOSENSORS & BIOELECTRONICS, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB, vol. 12, no. 7, 1997, pages 587-599, XP002113741 ISSN: 0956-5663 * abstract; figure 1 *	1-8	

-The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 9 April 2003	Examiner Komenda, P
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>& : member of the same patent family, corresponding document</p>			

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EP 01 30 8139

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1-8



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LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 01 30 8139

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-8
2. Claims: 9-13
3. Claims: 14-18

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 01 30 8139

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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09-04-2003

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0798561	A	01-10-1997	JP 9269325 A	14-10-1997
			CA 2188848 A1	01-10-1997
			EP 0798561 A1	01-10-1997
			US 5866321 A	02-02-1999

WO 9967628	A	29-12-1999	AU 4833899 A	10-01-2000
			EP 1090286 A1	11-04-2001
			JP 2002518998 T	02-07-2002
			WO 9967628 A1	29-12-1999
			US 2002081588 A1	27-06-2002

WO 9927355	A	03-06-1999	DE 19751658 A1	29-07-1999
			WO 9927355 A1	03-06-1999
			EP 1032826 A1	06-09-2000
			US 6458600 B1	01-10-2002

US 5945286	A	31-08-1999	NONE	

WO 9951778	A	14-10-1999	US 6221586 B1	24-04-2001
			AU 3550699 A	25-10-1999
			EP 1068359 A1	17-01-2001
			JP 2002510791 T	09-04-2002
			WO 9951778 A1	14-10-1999
			US 2002055103 A1	09-05-2002
			US 2002146716 A1	10-10-2002

EPO FORM P0459

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